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USE OF THE BEN FRANKLIN SUBMERSIBLE AS A SPACE STATION ANALOG

Volume IV – Microbiology
OSR-70-7

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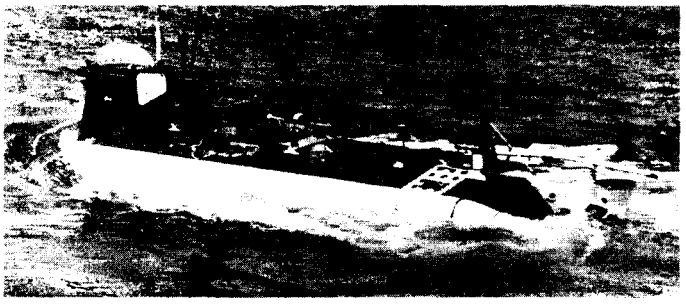
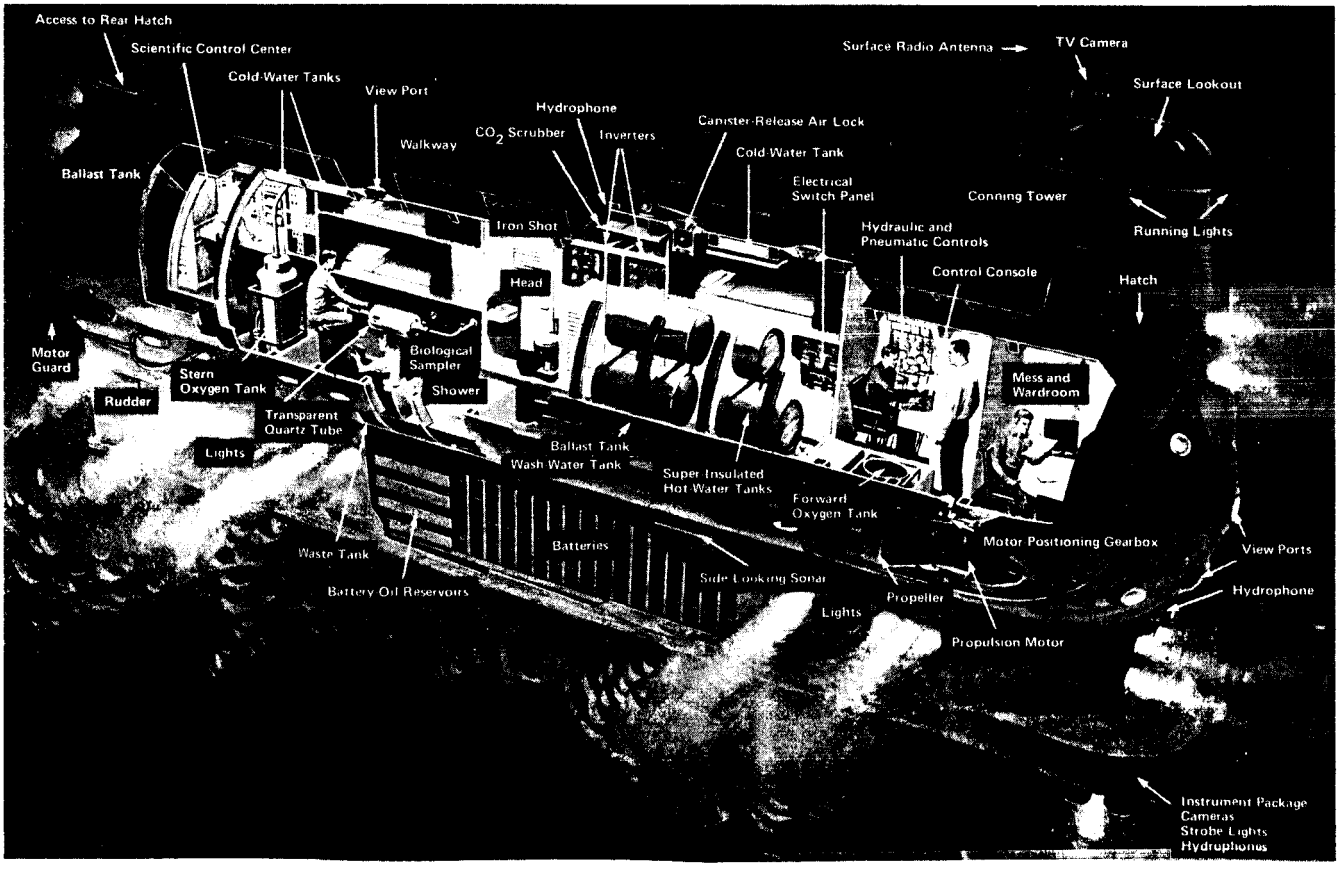
THE BEN FRANKLIN DURING THE GULF STREAM DRIFT MISSION





FOREWORD

During 1969, the Ocean Systems Department of Grumman Aerospace Corporation conducted the 30-day Gulf Stream Drift Mission, using the BEN FRANKLIN submersible. As a part of this mission, a NASA study was conducted to investigate man related activities which are analogous to long-duration space station missions. During the mission, a NASA crew member was aboard the BEN FRANKLIN for data collection, observation, and task participation. This work was performed in accordance with the Statement of Work in NASA Contract NAS 8-30172, "Use of BEN FRANKLIN as a Space Station Analog," for the George C. Marshall Space Flight Center, Advanced Systems Office, under the direction of C.B. May. The program was coordinated by Manager M. F. Markey of NASA, Washington Headquarters.

The Final Report consists of the following five volumes:

- OSR-70-4, Volume I, Summary Technical Report
- OSR-70-5, Volume II, Psychology and Physiology
- OSR-70-6, Volume III, Habitability
- OSR-70-7, Volume IV, Microbiology
- OSR-70-8, Volume V, Maintainability

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ABSTRACT

This report presents the NASA effort using the BEN FRANKLIN submersible as a space station analog during the 30-day Drift Mission in the Gulf Stream, starting July 14 and ending August 14, 1969. The areas of investigation include:

- Psychological and Physiological measurements during the pre-mission, mission, and post-mission phases
- Habitability in a closed ecosystem
- Microbiological evaluation of the water system, human flora, and environmental samples
- Maintainability considerations for scheduled and unscheduled tasks.

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VOLUME 4

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SECTION I

INTRODUCTION

The Gulf Stream Drift Mission (GSDM) of the BEN FRANKLIN during the summer of 1969 provided a unique opportunity of studying the interactions between the microbiological status of man, his environment, and his food and water. Unlike other studies of isolated groups, the subjects involved in this mission were undertaking a real mission under real stresses imposed by mission operations, physical confinement, and a hostile and dangerous environment.

Previous chamber experiments (Appendix A) such as those conducted at Boeing (Ref. 1 Section 5), Republic (Ref. 2), Wright Patterson Air Force Base (Ref. 3), Tektite (Ref. 4), and McDonnell Douglas (Ref. 5) were primarily designed to evaluate hardware or physiological parameters. The microbiological consideration was secondary; therefore frequent air lock operations and external life support equipment were permitted.

The GSDM was the first time an attempt was made to allow men to control their own microbiological condition through the application of antimicrobial agents as remedial measures. Despite some evidence that active microbial control in chamber studies is contra-indicated, vigorous, microbial control through the use of antimicrobial agents was attempted. This was tried to see whether in a truly closed system, results of such actions would confirm or reject conclusions arrived at in chamber studies. Also the basic emphasis on mission completion strongly influenced the decision to attempt microbial control through the priori decision to use antimicrobial agents. The microbiological study, therefore, was planned with the following objectives:

- On board monitoring of microbial contamination
- On board microbial control to prevent or reduce odors and prevent the spread of disease
- Preservation of microbial samples for base laboratory evaluation
- Routine use of germicides.

Although the GSDM provided a completely closed ecology with an unsophisticated life support system and a crew with a true operational goal, the characteristics of the BEN

FRANKLIN are similar to but not identical to those of a space craft. Areas of divergence include the water and waste management systems and the lack of a zero gravity environment.

The information collected permitted the identification of potential problem areas caused by total biological isolation in conjunction with the routine use of germicides, insight to the dynamics of the microbial ecology, evaluation of onboard monitoring by present methods and the evolution of recommendations for requirements in a closed ecology.

SECTION 2

EXPERIMENTAL APPROACH

The format of the GSDM microbiological effort is presented in Figure 2-1. The water was sampled daily with the exception of Days 12, 28, 29, and 30. Human and environment samples were taken every third day. Notations as to washing procedures, germicide treatment, temperature, and humidity are presented with the format for comparison purposes. Figures 2-2 and 2-3 indicate the body and environmental areas sampled. A total of 1530 samples were taken, of these 1475 were for the microbiology study. This resulted in approximately 2230 isolates which required an estimated 15,000 culturing steps for identification to genus.

2.1 PRIMARY SAMPLING

2.1.1 Human

2.1.1.1 Body Surfaces

The axilla, groin, forehead, and back of neck were sampled by using Rodac® contact plates containing blood agar. The procedure was to carefully remove the cover, place the plate in contact with the skin to replicate any organisms present, remove, recover, label, tape and store the plate at ambient temperature. These plates provided both qualitative and quantitative data.

2.1.1.2 Other Body Areas

The nose, throat, ears, and toes were sampled with sterile swabs which were preserved for post-mission analysis by insertion into screw-capped vials of Cary-Blair transport medium. The vials were stored at ambient boat temperature. These provided qualitative data only.

2.1.2 Environment

2.1.2.1 Surfaces

The surfaces were sampled by using Rodac plates in a similar manner as in body surfaces. Instead of blood agar, Letheen agar was used to neutralize any residual antimicrobial activity arising from washing the surfaces with germicide.

DATE 1969 MISSION DAY	7-7 -7	7-9 -5	7-11 -3	7-12 -2	7-13 -1	7-14 0	7-15 1	7-16 2	7-17 3	7-18 4	7-19 5	7-20 6	7-21 7	7-22 8	7-23 9	7-24 10	7-25 11	7-26 12	7-27 13	7-28 14	7-29 15	7-30 16	7-31 17
WATER																							
SYSTEM STERILIZED				X																			
PUMPED OFF					X																		
LOADED					X																		
SAMPLED						X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
FILTER CHANGED													X								X		
TANK IN USE					#1			#2											#3				
HUMAN																							
SAMPLING		X	X			X		X			X			X			X			X			X
GARMENT CHANGE																							
UNDER									X			X			X			X			X		
OUTER/LINEN													X										
WASHING	AT VARYING TIMES THROUGHOUT MISSION WITH ANTIBACTERIAL SOAP "SAFEGUARD"																						
ENVIRONMENT																							
SAMPLING AIR				X				X			X			X			X			X			X
SURFACES	X			X				X			X			X			X			X			X
WASHING SURFACES						X							X							X (0400)			
DISHES	DAILY DIP WITH QUATERNARY AMINE SANITIZER															2X DAILY							
SPRAY GARBAGE	DAILY THROUGHOUT MISSION																						
WASTE																							
WELLODYNE	WITH EACH FLUSH, 1-OZ "WELLODYNE" (AUTO FEED)																						
MICROGARD	4 OZ PER TANK INITIAL CHARGE +2 OZ EVERY THIRD DAY →																						
TEMP (°F)							55-65	65	55	62	67	62	53	66	53	65	72	84	75	65	67 →		
REL HUMIDITY (%)	70-80 →								82	70-80 →			82	82	82	70-80	62	70-80					

Shower Drain Plugged

Shower drain leaked into Bildge

Replaced purafil in head blower

Figure 2-1. Experimental Procedures (Sheet 1 of 2)

DATE 1969 MISSION DAY	8-1 18	8-2 19	8-3 20	8-4 21	8-5 22	8-6 23	8-7 24	8-8 25	8-9 26	8-10 27	8-11 28	8-12 29	8-13 30	8-14 31	8-16 +2	8-18 +4	8-22 +8	8-26 +12
WATER																		
SYSTEM STERILIZED																		
PUMPED OFF																		
LOADED																		
SAMPLED	X	X	X	X	X	X	X	X	X	X							X	
FILTER CHANGED																		
TANK IN USE																		
HUMAN																		
SAMPLING	X		X			X			X			X			X	X		X
GARMENT CHANGE																		
UNDER				X			X			X				X				
OUTER/LINEN				X							X							
WASHING																		
ENVIRONMENT																		
SAMPLING AIR				X			X										X	
SURFACES				X			X			X							X	
WASHING SURFACES				X Galley Head Shower		} DAILY WITH QUAT →												
DISHES																		
SPRAY GARBAGE																		
WASTE																		
WELLODYNE	Extra 4 oz. ←			2 oz. Per Day →														
MICROGARD																		
TEMP (°F)	→							65	64	68	67	64	68					
REL HUMIDITY (%)	70	74	77	75	77	75	73	77	77	71	72	77	73					
													X					

Figure 2-1. Experimental Procedures (Sheet 2 of 2)

LOCATIONS	PRE- AND POST-MISSION	DURING MISSION	METHOD
Nose	X	X	Swab and Cary Blair Transport
Throat	X	X	Swab and Cary Blair Transport
Rt. Ear	X	X	Swab and Cary Blair Transport
Lt. Ear	X		Swab and Cary Blair Transport
Rt. Foot	X	X	Swab and Cary Blair Transport
Lt. Foot	X		
Forehead	X	X	Blood Agar Rodac Plate
Back of Neck	X		Blood Agar Rodac Plate
Rt. Axilla	X	X	Blood Agar Rodac Plate
Lt. Axilla	X		Blood Agar Rodac Plate
Rt. Groin	X	X	Blood Agar Rodac Plate
Lt. Groin	X		

Figure 2-2. Human Body Sampling Locations

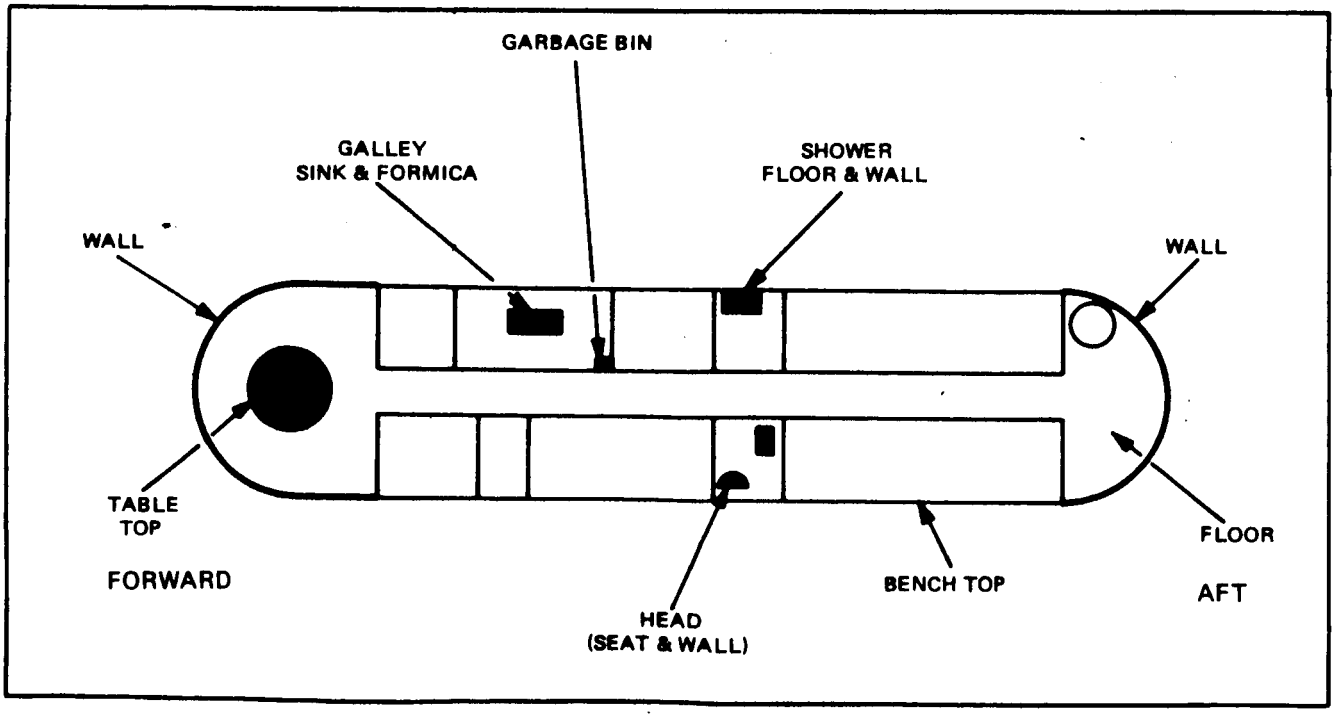

Figure 2-3. Environment Sampling Locations

2.1.2.2 Air

The air was sampled using a six-stage Andersen sieve sampler with petri dishes containing nutrient agar. After sampling 5 cubic feet of air, the plates were removed, sealed, and stored at ambient boat temperature.

2.1.2.3 Sorbents

Various bags of sorbents returned to Grumman at the end of the mission were cultured for a qualitative estimate of microorganisms present by the use of Rodac contact plates, or by shaking the bags over blood and Letheen agar plates.

2.1.3 Water and Waste Water

During the mission potable water was sampled using Millipore Field Monitor kits, according to the procedures recommended by the Millipore Corporation, except that the monitors were incubated at ambient temperature ($\approx 67°F$) rather than the specified 95°F.

Endo medium (for coliforms), Total medium, and Yeast-mold medium were used for each water sample. Monitors were observed at 24, 48, and 72 hours for the presence of growth and then stored unopened until the end of the mission.

Provision was made for iodine concentration determination using a Hach color comparator kit.

No waste water samples were taken during the mission.

Pre- and post-mission water and waste water were collected in sterile polypropylene bottles and returned to the Grumman biotechnology laboratory for analysis according to USP sterility test (Ref. 6), standard plate count, and chemical analysis (Ref. 7).

2.1.4 Food

Selected packs of those foods which had been repackaged from bulk supplies were cultured according to selected Food and Drug Administration procedures to determine the total bacterial count and coliforms. Pour plates of Tryptone-Glucose Extract (TGE) agar were used for total count and desoxycholate lactose agar for coliforms. Dilutions of the foods were made in sterile distilled water.

2.1.5 Garments and Linen

All garments and linen were stored onboard until the end of the mission and then returned to Grumman biotechnology laboratory. Selected items, which had been stored

in closed plastic bags, were sampled using Letheen agar Rodac contact plates in the same manner as for the environment and body surfaces. Plates were incubated at 95° F for 48 hours and then processed for identification of organisms to genus.

All samples taken during the mission were incubated and stored at ambient boat temperature until the conclusion of the mission when they were returned to the Grumman Biotechnology lab for further analysis.

Onboard, when possible, total counts were made at 24, 48 and 72 hours for Rodac and Andersen plates.

2.2 SECONDARY CULTURING

All secondary culturing was accomplished at the Grumman biotechnology laboratory.

2.2.1 Rodac Plates

2.2.1.1 Bacteria

All colonies on Rodac plates were counted without differentiation of colony types to provide the "total count" figures. (In generating averages, results too numerous to count (TNTC) were taken as 1000). From these plates, representative colonies of each morphological type were described, picked to Brain Heart Infusion Broth (BHI), and incubated at 95° F for 24 hours. From these broths, gram stains and transfers to tryptic soy agar slants were made. Gram stained slides were observed microscopically. The slants were incubated at 95° F for 24 hours and then refrigerated.

2.2.1.2 Fungi

All fungal colonies were transferred to mycophil agar slants, incubated 5 days at 77° F and then refrigerated.

2.2.2 Swabs

Each swab was removed from its Cary-Blair transport medium with flamed forceps, streaked into two blood agar plates, and then inserted into a tube of slanted mycophil agar. The blood agar plates were incubated at 95° F for 24 hours, one aerobically, and one anaerobically using the BBL Gas Pak System. Mycophil slants were incubated at 77° F for 5 days and then refrigerated.

From the blood agar plates, representative colonies were described, picked to BHI broth, incubated at 95° F for 24 hours, then gram stained and transferred to agar slants which were incubated at 95° F for 24 hours. These slants were then stored in the refrigerator. Gram stained slides were observed microscopically.

2.2.3 Andersen Plates

Total counts of atmospheric samples were made for each plate. Representative colonies were described, picked, and processed as for Rodac plates.

2.2.4 Millipore Field Monitors

From the Endo and Total monitors, growth was transferred to BHI broth and processed as above. From the Yeast-Mold monitors, growth was transferred to Mycophil agar slants and processed as for fungi above.

2.3 IDENTIFICATION TO GENUS

2.3.1 Bacteria

All bacterial isolates were further processed by standard diagnostic procedures (Ref. 9) for identification to genus. A schematic diagram is presented in Figure 2-4.

2.3.2 Fungi

Identification of fungi to genus was based on colonial and microscopic morphology using lacto-phenol cotton blue staining fluid.

2.4 LIMITATIONS

Several conditions peculiar to the GSDM had a direct bearing on the data obtained:

- Limited storage space dictated the amount of media which could be taken aboard and thereby directly affected the frequency of sampling.
- Limited power precluded having an incubator aboard. Lack of the incubator favored the recovery of hardy organisms that could survive better in lower temperatures and also inhibited the recovery of fastidious human organisms which are very sensitive to temperature.
- Length of the mission necessitated storage of samples for up to 4 weeks at ambient temperatures, with longer holding times before culturing. Realizing that this would affect the viability of many organisms, the decision was made to process the latest samples first expecting to obtain good recovery on those and hoping that the others would remain viable. To work with the oldest samples first would have resulted in all isolation work being done on deteriorating material.
- The data obtained were analyzed by making comparisons of the total counts and by analysis of the absolute rather than relative presence of a particular organism. It is more common to make these analyses by evaluating the relative

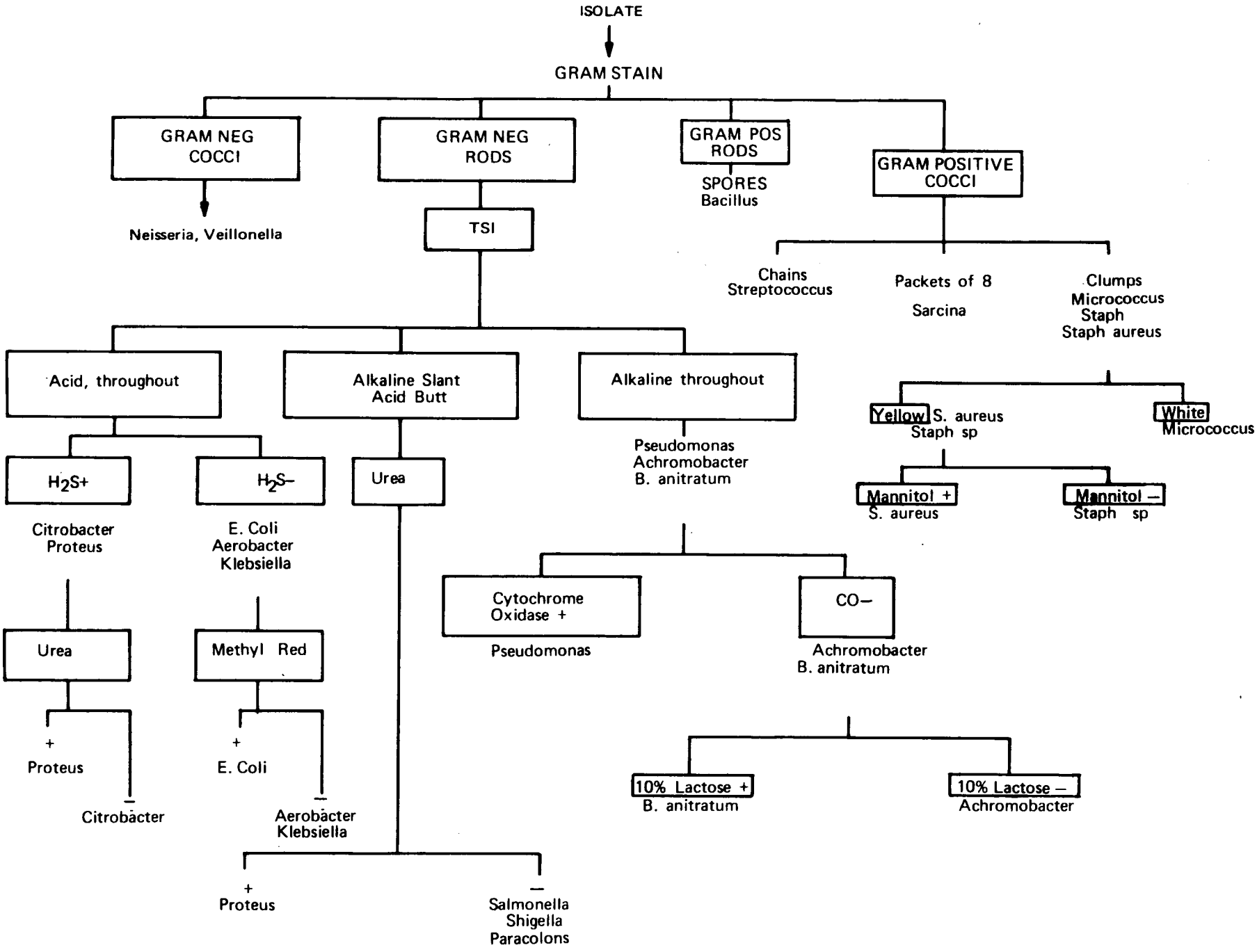

Figure 2-4. Bacteria Identification Schematic

frequency of organisms. However, this approach was impossible, since no differential counts were made. Therefore, in presentation of the data, the following definitions apply:

- Shift: Change in ratio (qual) of one group of organisms to another (i.e. gram positive vs. gram negative bacteria)
- Simplification: Reduction in the no. of different types (genera) of microorganisms recovered
- Incidence of Genera: Sum of each genus isolated multiplied by no. of times isolated
- Number of Total Isolates: Sum of all microorganisms recovered
- Number of Different Genera: Tabulation of types (genera) isolated taking each genus as one

2.5 SPECIAL CONDITIONS

Microbial control through the prophylactic use of antimicrobial agents was attempted during the GSDM. The agents and areas of use are as follows:

- Potable Cold Water. Tincture of iodine was used in the potable cold water system based on the Apollo LM experience. Sterilization of the system was attempted with a 75 ppm solution. Provision was made for reiodization of the water to maintain a 7.5 ppm residual level of iodine. However, the reiodization procedure was not followed, because the crew objected to the taste of iodine in their potable supply
- Waste Tanks. Wellodyne, a combination of iodine and phosphoric acid, was used for the biocidal properties of iodine and low pH produced by phosphoric acid which inhibitis the growth of microorganisms and also prevents ammonia production. This procedure was supplemented by the addition of the quaternary amine antimicrobial Micrograd
- Environment. Surface cleaning was effected with a Microgard solution according to the schedule in Figure 2-1. Garbage was sprayed with Microgard and dishes were dipped daily in a quaternary amine sanitizer

ANTIMICROBIAL AGENTS

PRODUCT	USE & APPLICATION	ACTIVE INGREDIENTS	CONCENTRATION	
			AS SUPPLIED	AS USED
Tincture of Iodine	1. Sterilization of Water System 2. Potable Cold Water Additive	Iodine, Ethyl Alcohol	8.4 grams/litre 0.84 grams/litre	75 ppm 7.5 ppm
Wellodyne West Chem Prods. L.I. City, N.Y.	Waste Tanks (1 oz per flush, automatic dispenser)	Iodine, Phosphoric Acid (H_3PO4)	I_2 - 1.75% H_3P04 - 15.95%	Full Strength
Safeguard Soap Proctor & Gamble Cincinnati, Ohio	All personal hygiene	3,4,5 Tribromosalicylamide 4' Dichloro - 3 (Trifluoro-methyl) Carbanilide, 3,4,4' Trichlorocarbarilide	-	As Supplied
Microgard DD	Dish Sanitizer - Daily Dip	Alkyl Dimethyl Benzylammonium cl^- Alkyl Dimethyl Ethyl Benzyl-ammonium cl^-	24.35% Active	1 oz/gal
GS	1. Garbage Spray, Cleaner 2. Waste Tank (2 to 4 oz/ application)	Same as DD plus n-tributyltin salt	28.76% Active	1. 1 oz/gal 2. Full Strength
GL	Garments and Linen	Same as GS	30.75% Active	0.1% by Weight

Figure 2-5. Antimicrobial Agents Used

- Garments and Linen. These were treated only once, prior to the mission, with a Microgard solution by Micron Clean Uniform Service, Newburgh, New York
- Personal Hygiene. All body washing was done with Safeguard soap.

A summary of the antimicrobials used is presented in Figure 2-5.

SECTION 3

AREAS OF INVESTIGATION

3.1 HUMAN FLORA

Within the constraints of the mission and sampling protocol as outlined in Subsection 4.2, intensive culturing of the samples taken from the crew members was attempted to obtain as good a profile as possible of the changes occurring during total isolation.

One week was available for pre-mission testing. Three sets of samples were obtained from most crew members, unfortunately under uncontrolled conditions. Post-mission sampling also suffered from lack of controlled conditions and the absence of samples for the first 2 days after the end of the mission. During the hectic pre-mission and post-mission activities, all crew members were not available for simultaneous sampling.

The effects of body washing cannot be evaluated because there were no specific washing schedules. In an attempt to reduce variability induced by the garments, all samples were taken just prior to change into clean garments; that is, after 5 days of wear.

3.1.1 Total Count

Total counts for the body areas sampled with Rodac plates (groin, axilla and forehead) are presented in Figure 3-1. Large differences occur between on-board and base laboratory readings of mission samples with the on-board readings invariably lower. Since the effect of low temperature incubation is to retard the growth and replication of microorganisms, many colonies might not have been visible at 72 hours. Reinforcing this opinion is the fact that on Day 11 of the mission when the boat's temperature rose about 10 degrees, the on-board counts approached those of the base laboratory (Figure 3-2).

A second influence would be that of temporary bacteriostasis. Residuals on the skin from the antimicrobial soap used for washing or from the antimicrobial impregnated garments could transfer along with the microorganisms onto the Rodac plates. Since

TOTAL MICROBIAL COUNTS (PER 4 SQ. IN. BLOOD RODAC PLATES)

CREWMAN 1

MISSION DAY	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Axilla - L (Lab)	NC	NC	7											47	11	27
R (Lab)	NC	NC	23	8	0	33	130	6	231	44	20	3	250	61	9	39
(O/B)				0	0	0	98	4	2	2	3	2				
Groin - L (Lab)	NS	NC	107											TNTC	HS	6
R (Lab)	NS	NC	94	TNTC	3	1	27	11	13	200	150	75	300	74	NS	8
(O/B)				0	0	0	10	3	5	0	4	7				
F Head (Lab)	NC	NC	>700	300	300	88	100	TNTC	TNTC	TNTC	TNTC	200	TNTC	TNTC	700	500
(O/B)				2	0	0	38	9	5	40	38	20				
Back of Neck (Lab)	NC	NC	36											TNTC	800	250

CREWMAN 2

Axilla - L (Lab)	NS	NGOT	>500											7	20000	1000
R (Lab)	NS	NGOT	>800	17	45	2	170	8	TNTC	15	55	100	TNTC	119	42	1000
(O/B)				0	0	0	113	1	0	0	20	75				
Groin - L (Lab)	NC	NC	>200											63	25	11
R (Lab)	NC	NC	146	TNTC	30	6	50	14	115	18	40	54	150	TNTC	26	24
(O/B)				0	8	1	10	6	0	0	7	3				
F Head (Lab)	NS	NGOT	NGOT	200	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	300	TNTC	300	300	95
(O/B)				2	14	10	TNTC	TNTC	10	TNTC	TNTC	50				
Back of Neck (Lab)	NS	NGOT	350											64	120	96

Legend: L = Left
R = Right
Lab = Count after plates returned to Grumman.
(O/B) = On board 72 hour reading.
NS = No sample.
NGOT = No growth on transfer.
NC = No count.
$NC_{(S)}$ = No count due to spreader.
TNTC = Too Numerous to Count

Figure 3-1. Total Microbial Counts (Sheet 1 of 3)

TOTAL MICROBIAL COUNTS (PER 4 SQ. IN. BLOOD RODAC PLATES)

CREWMAN 3

MISSION DAY	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Axilla - 1 (Lab)	NC	NS	>300											200	500	1
R (Lab)	NC	NS	>300	5	57	2	150	5	200	57	TNTC	40	110	79	186	74
(O/B)	0	20	8	>150	0	7	1	TNTC	7							
Groin L (Lab)	NC	NS	>1000											TNTC	98	30
R (Lab)	NC	NS	>500	100	31	87	50	130	106	300	30	200	NC(S)	42	110	34
(O/B)				5	30	0	23	8	10	15	12	0				
F Head (Lab)	NC	NS	TNTC	TNTC	TNTC	TNTC	TNTC	300	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
(O/B)				0	5	0	TNTC	>600	3	5	8	20				
Back of Neck (Lab)	NC	NS	48											500	TNTC	95

CREWMAN 4

Axilla L (Lab)	200	NC	>500											TNTC	500	TNTC
R (Lab)	200	NC	>1000	28	13	5	14	90	85	55	30	15	TNTC	TNTC	500	TNTC
(O/B)				0	0	0	20	60	4	16	1	0				
Groin L (Lab)	109	NC	>500											300	300	30
R (Lab)	152	NC	>400	NS	15	54	70	35	63	80	60	250	71	500	144	18
(O/B)				9	0	1	30	5	7	20	0	0				
F Head (Lab)	15	NC	38	40	30	100	>300	70	19	100	64	100	90	NC	50	4
(O/B)																
Back of Neck	3	NC	9											200	123	21

Figure 3-1. Total Microbial Counts (Sheet 2 of 3)

TOTAL MICROBIAL COUNTS (PER 4 SQ. IN. BLOOD RODAC PLATES)

CREWMAN 5

MISSION DAY	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Axilla - L (Lab)	48	NC	75											68	25	26
R (Lab)	158	NC	70	TNTC	TNTC	300	200	115	200	200	75	TNTC	72	300	18	22
(O/B)				0	25	0	>100	13	12	0	0	6				
Groin L (Lab)	TNTC	NC	>200											56	24	28
R (Lab)	TNTC	NC	>300	28	200	135	23	90	50	300	140	180	13	100	24	15
(O/B)				0	0	0	13	3	1	3	0	0				
F Head (Lab)	500	NC	TNTC	TNTC	NC(S)	TNTC	TNTC	TNTC	300	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
(O/B)				TNTC	50	1	TNTC	35	17	4	8	3				
Back of Neck	21	NC	81											400	150	165

CREWMAN 6

Axilla L (Lab)	NC	TNTC	TNTC											TNTC	300	200
R (Lab)	NC	TNTC	TNTC	23	31	10	30	200	105	100	300	150	15	TNTC	75	175
(O/B)				2	0	0	0	30	0	0	21	0				
Groin L (Lab)	NC	TNTC	TNTC											TNTC	87	38
R (Lab)	NC	TNTC	TNTC	300	300	75	TNTC	TNTC	300	TNTC	TNTC	300	25	TNTC	66	102
(O/B)				2	0	0	>50	20	10	TNTC	>100					
F Head (Lab)	NC	TNTC	>100	300	30	100	200	300	200	300	300	TNTC	200	TNTC	125	49
(O/B)				75	4	0	100	5	5	0	26	17				
Back of Neck	NC	TNTC	75											TNTC	76	2

Figure 3-1. Total Microbial Counts (Sheet 3 of 3)

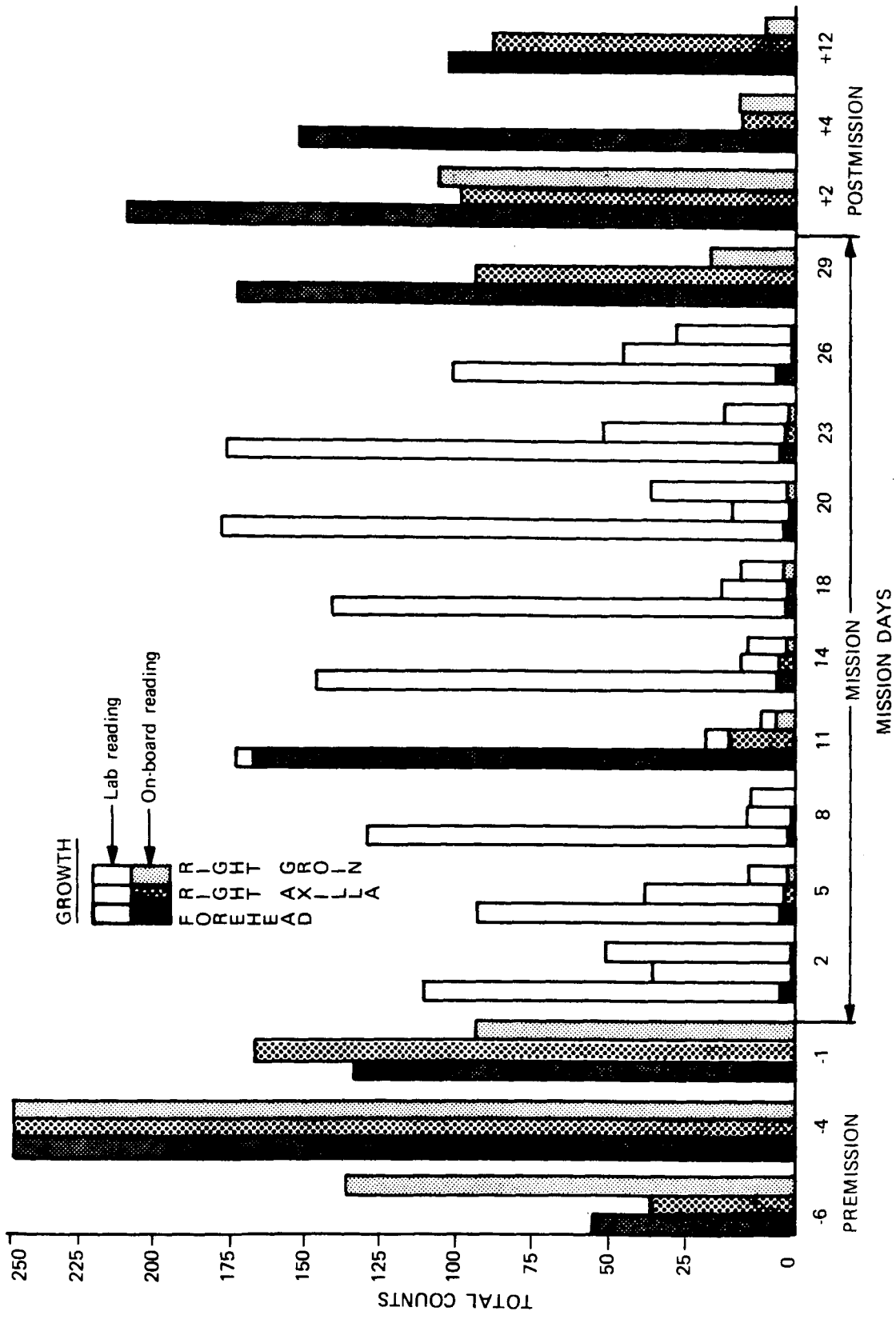

Figure 3-2. Human Flora Total Counts: Comparison On-Board and Lab Reading

blood agar was used in the hope of recovering the more fastidious bacteria (instead of a neutralizing medium such as Letheen Agar), the potential carryover helped to delay appearance of colonies.

Regarding the number of organisms found on the various body areas, a wide range of individual variation existed. Plotting an average of the six men for the axilla, the groin and the forehead (Figure 3-3) showed some trends. However, when interpreting these trends, one must keep in mind the great range between maximum and minimum values at each point. (Figure 3-1) In general, after an initial drop in number of organisms, an increasing trend was noted, with the exception of the groin samples, after Day 20 of the mission. Mission results indicate that counts on the forehead were far above those for either the groin or the axilla, a phenomenon which cannot be explained. Since conditions of sampling and storage were the same for all Rodac plates taken during the mission, it is possible that the garment treatment played a direct role in these relative differences. While a reduction in microbial population may decrease body odor, the overall effect on the balanced skin ecology can be detrimental and may greatly outweigh any possible advantages of diminishing odor. In fact this may account for the appearance of numerous skin rashes (Subsection 3.6). Looking at each determination individually (Figure 3-2), a rise in microbial populations is noted on Day 11. This corresponds with the rise in temperature in the boat.

3.1.2 Simplification/Shift of Flora

Another aspect of the human flora analysis was directed at overall shifts and/or simplification of flora. For this, information on all human samples, both those taken by Rodac plates and with swabs, was utilized. These data are presented in Figures 3-4 through 3-9.

Since many diverse microorganisms contribute to the microbial profile of a particular locale or individual, it is the maintenance of a balance which may be the key to health and well-being. This balance is maintained by the competitive interaction of organisms themselves and between bacteria and the environment. One of the strongest factors enabling the body to resist invasion by undesirable microbes is the competitive inhibition of the "normal" or indigenous flora. Alterations can potentially lead to several undesirable results:

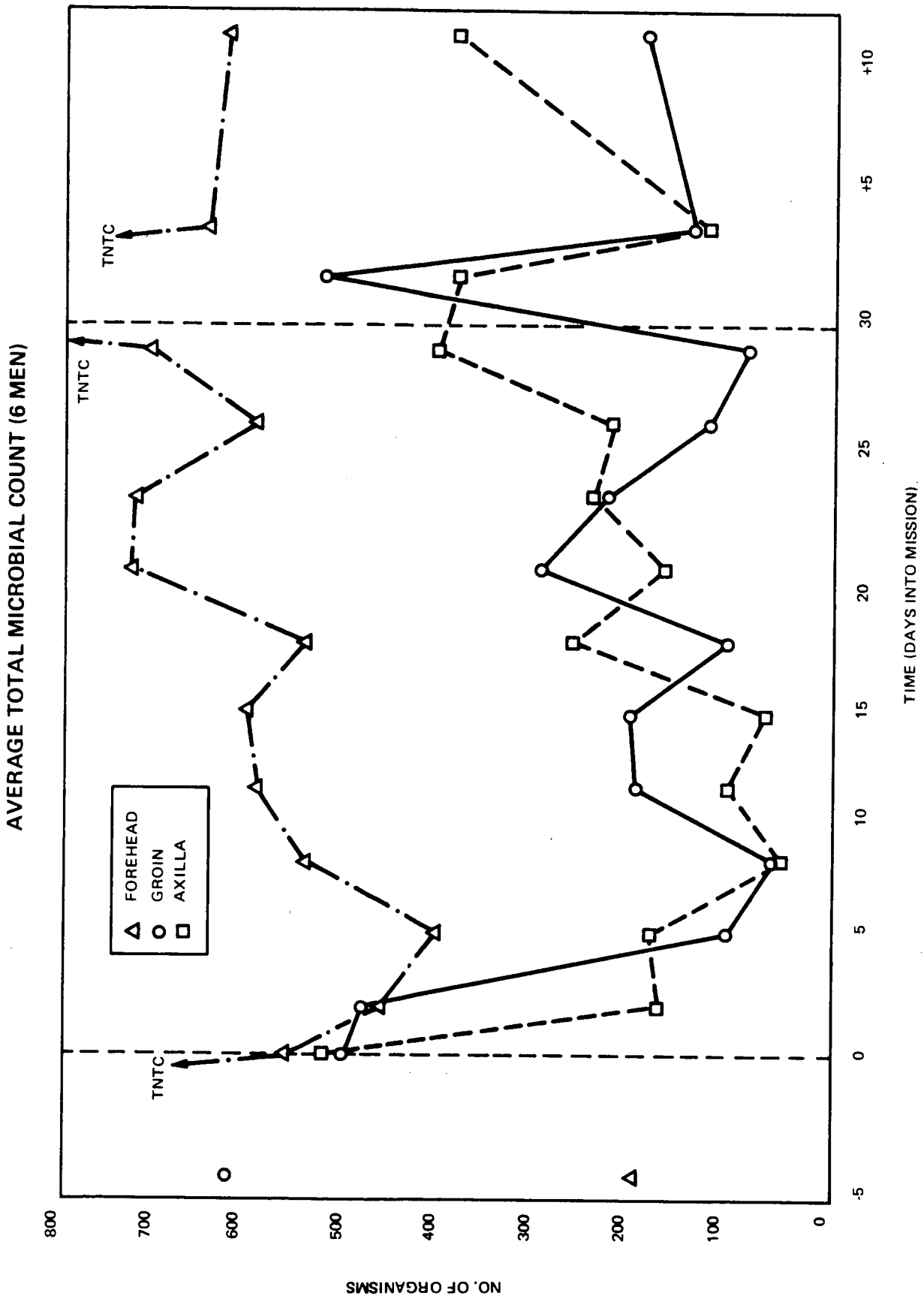

Figure 3-3. Averaged Total Microbial Count: Forehead, Axilla, Groin

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Days	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Date Sample PD	7/9/69 1	7/11/69 2	7/14/69 3	7/16/69 4	7/19/69 5	7/22/69 6	7/25/69 7	7/28/69 8	7/31/69 9	8/3/69 10	8/6/69 11	8/9/69 12	8/12/69 12a	8/16/69 13	8/18/69 14	8/26/69 15
Nose	Pseudo E. coli	Microc E. coli	β-Strep Bac	Microc E. coli	E. coli	E. coli	Microc	E. coli	Microc	S. aureus	S. aureus	E. coli	Microc	E. coli	E. coli	Microc
Throat	Pseudo	Prot Aerob	Pseudo β-Strep α-Strep	Pseudo	E. coli	Pseudo	Microc	Pseudo	α-Strep	Microc	S. aureus	Aerob	Aerob	α-Strep Microc	α-Strep	α-Strep
Rt. Ear	Microc	Microc Mold	Bac Candida	NGOT	NGOT	Candida	Aerob Bac	Aerob Candida	Pseudo	Candida	Candida Bac	Candida	Microc	Microc	Microc	Microc Bac Rhodo
Lt. Ear	NS	Microc	Microc Candida Coryne											Microc	Microc	Microc
Rt. Foot	Microc	Microc Rhodo	NGOT	Microc	Microc	Microc Candida Rhodo	Microc	Aerob	Microc	Microc	Aerob Candida	Microc	Microc	Microc	Microc	Microc Rhoda
Lt. Foot	Pseudo Rhodo	Microc	NS											Microc	Microc Rhodo	Aerob
Forehead	Bac Microc Sarc	Microc	Microc Sarc	Microc	Microc	Microc Bac	Microc Bac	Microc Aerob B. anit	Microc B. anit	Microc	Bac Microc	S. aureus	Microc	Microc Bac	Microc	Microc B. anit
Back Of Neck	Bac Microc	Microc	Microc Bac Sarc											Bac	Microc	Microc
Rt. Axilla	Microc	Microc	Microc Bac	Microc		Microc	Microc Aerob	Microc	B anit	B. anit Microc	Aerob Microc	Microc	Pseudo Microc	α-Strep Bac Coryne	NGOT	Microc Aerob
Lt. Axilla	Microc Sarc	Microc	Microc Sarc											Microc Coryne	Bac Microc	Bac
Rt. Groin	NS	Bac Sarc	Microc Sarc	Microc	Candida	Microc	Microc	Microc Candida	Microc Pseudo	Microc	Aerob Bac Microc	Microc	Microc	NGOT	NS	NGOT
Lt. Groin	NS*	Microc	Microc											Microc Coryne	NS	Microc

D23

ABBREVIATIONS

Micrococcus = Microc
Bacillus = Bac
Sarcina = Sarc
Pseudomonas = Pseudo

Aerobacter = Aerob
Proteus = Prot
Rhodotorula = Rhodo
Corynebacterium = Coryne

Citrobacter = Citro
B. anitratum = B. anit
Trichophyton = Tricho
Alternaria = Altern

Achromobacter = Achromo
Aspergillis = Asperg.
NGOT = No Growth On Transfer
NS = No Sample

Figure 3-4. Organisms Recovered From Human Samples, Man 1

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Days	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Date	7/9/69	7/11/69	7/14/69	7/16/69	7/19/69	7/22/69	7/25/69	7/28/69	7/31/69	8/3/69	8/6/69	8/9/69	8/12/69	8/16/69	8/18/69	8/26/69
Nose	Microc Coryne	Microc Coryne	Microc Coryne	Microc	Microc	Microc	Microc	Aerob	Microc	Microc	Prot	Aerob	Aerob	Microc	Microc Bac	Microc
Throat	α-Strep	α-Strep	α-Strep	NGOT	α-Strep	NGOT	α-Strep	Aerob	α-Strep	α-Strep	α-Strep	α-Strep	Aerob	Microc α-Strep	α-Strep	α-Strep
Rt. Ear	Microc Rhodo	Pseudo	Aerob	Microc Bac	NGOT	Microc Rhod	Microc	Aerob	Microc	Aerob	Aerob	Aerob Microc	Aerob	Aerob	Aerob Microc	Aerob
Lt. Ear	NGOT	Microc Coryne	Aerob											Microc	Microc B. anit Asperg	Aerob
Rt. Foot	Microc	Aerob	Candida Aerob	Citro	Pseudo Prot	Prot Candida	Microc Candida	Microc Tricho	Aerob	Candida	Candida B. anit	Candida	Aerob Candida	Aerob	Microc	Microc Rhodo
Lt. Foot	Pseudo	NS	Pseudo Prot											Prot	Coryne	Aerob Pseudo
Forehead		NGOT	NGOT	Microc Bac S. aureus	Microc	Microc Aerob	Bac Aerob	Bac Aerob	Microc	Microc	Microc	Aerob	NGOT	Aerob	Microc	Microc Aerob
Back of Neck		NGOT	Microc Sarc Mold											Microc	Microc B. anit	Microc
Rt. Axilla		NGOT	Microc Sarc	Microc	Microc Bac	Microc Asperg	Microc	Bac	Microc	Microc	Microc	Aerob	Aerob B. anit	Microc	Microc	Microc
Lt. Axilla		NGOT	Microc Sarc											Sarc	Microc	Bac
Rt. Groin	Microc Bac Sarc	Sarc	NGOT	Sarc Microc	Microc	Microc	Sarc Microc	Aerob B. anit	Microc	Microc	Microc Aerob	Microc Aerob	B. anit	Microc	Microc	NGOT
Lt. Groin	Bac Sarc	Sarc	Microc Sarc											Microc Bac	Microc	Microc

Figure 3-5. Organisms Recovered From Human Samples, Man 2

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Days	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Date	7/1/69	7/11/69	7/14/69	7/16/69	7/19/69	7/22/69	7/25/69	7/28/69	7/31/69	8/3/69	8/6/69	8/9/69	8/12/69	8/16/69	8/18/69	8/26/69
Nose	Microc	↑	Microc	Microc	Microc	Microc	Microc	Pseudo	Aerob	Microc	Aerob	Aerob	Aerob	Microc	Microc Coryne	Microc
Throat	α-Strep		β-Strep	β-Strep Microc	β-Strep α-Strep	β-Strep Microc	β-Strep α-Strep	β-Strep	β-Strep	β-Strep	β-Strep	Aerob	β-Strep	β-Strep α-Strep	α-Strep Microc	α-Strep Microc
Rt. Ear	Rhodo		Microc Bac	Microc	Microc	Microc Rhodo	Aerob	Aerob	Aerob	Aerob	Aerob	Pseudo	Aerob Pseudo	Microc Candida	Microc	Altern
Lt. Ear	Candida		Bac Candida											Microc Candida	Microc	Bac Candida
Rt. Foot	Microc Candida		Pseudo Candida	Coryne Microc	Prot	Prot Rhodo Candida	Aerob Pseudo	Prot	Aerob		Achromo	Microc	Achromo Rhodo	Microc Altern	Microc Altern Tricho	Bac Tricho
Lt. Foot	Microc Candida Bac Mold	No	Pseudo Candida											Microc Candida	Microc	Bac Microc
Forehead	Microc	Samples	Bac	Microc	Microc	Microc	Microc	Bac Aerob	Microc	Microc	Bac Microc Aerob	Bac	Microc Pseudo Bac	NGOT	Microc	Microc
Back of Neck	Microc Sarc		Microc Sarc Bac											Microc	Microc	Bac Microc
Rt. Axilla	Microc Asperg		Coryne Bac	Microc	Microc	Microc	Bac	Sarc Aerob	Microc B. anit	Microc *Pseudo* Bac	Aerob	Aerob *Pseudo* Microc	Microc	Microc	Microc	Microc
Lt. Axilla	Microc Mold		Microc Sarc Bac											Microc	Microc	Microc
Rt. Groin	Microc Sarc		Microc Sarc Mold	Microc	Microc	Microc	Microc	Microc Bac	Microc Bac	Microc Bac	Microc Achromo	Microc	Bac	Sarc Microc Coryne	Microc	Microc
Lt. Groin	Microc Sarc	↓	Sarc Bac											Coryne Microc	Microc	Microc

Figure 3-6. Organisms Recovered From Human Samples, Man 3

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Day Date	-5 7/9/69	-3 7/11/69	0 7/14/69	2 7/16/69	5 7/19/69	8 7/22/69	11 7/25/69	14 7/28/69	17 7/31/69	20 8/3/69	23 8/6/69	26 8/9/69	29 8/12/69	+2 8/16/69	+4 8/18/69	+12 8/26/69
Nose	Microc	Microc Coryne Bac	Microc	Microc	S. aureus Microc	Microc	S. aureus	Aerob	Microc	Aerob	Aerob	Aerob	Aerob	Pseudo	Microc	Microc
Throat	β-Strep α-Strep	β-Strep α-Strep	β-Strep α-Strep Aerob	α-Strep	α-Strep β-Strep	α-Strep	α-Strep	Bac	Aerob	Pseudo	Microc	Aerob	Bac	Microc	Microc β-Strep	Microc α-Strep
Rt. Ear	Microc	Microc	Microc Bac	NGOT	Microc	Microc Asperg	Microc	Yeast	Pseudo	Aerob	Microc	Aerob	Aerob	Microc	Microc Altern	B. anit Microc Altern
Lt. Ear	Microc	Microc Sarc Mold	Microc											Microc	Microc	Microc
Rt. Foot	Microc Prot	Prot	Prot	Prot Pseudo	Prot	NS	Prot	Prot	NS	Prot	Prot	Prot	Aerob	Prot	Microc Candida	Prot
Lt. Foot	Microc	Microc Sarc	Prot Candida Mold											Prot	Prot Candida	Prot
Forehead	Microc Bac	Microc	Microc Sarc	Microc	Microc	Microc Asperg	Microc	Microc Aerob	S. aureus	Microc	Aerob Pseudo Achromo	Microc Aerob	Microc	Bac B. anit	Microc	Bac Microc
Back of Neck	NGOT	Microc Sarc												Bac Microc	Microc	NGOT
Rt. Axilla	Microc	Microc	Microc	Microc Sarc	Sarc	B. anit	Microc B. anit	Aerob Bac	Microc	Bac	Microc	Microc	Microc	B. anit	Microc	Microc
Lt. Axilla	Microc	NGOT	Microc A. niger											Coryne Microc B. anit	Microc	Microc
Rt. Groin	Microc Sarc	Microc	Microc Sarc	NS	Microc	Microc	Bac Microc	Aerob B. anit	Microc Aerob	Bac Coryne	Microc Pseudo Achromo	Microc	Microc B. anit	Coryne Microc	Microc	B. anit Microc
Lt. Groin	Microc Sarc Bac	Microc	Microc Sarc											Microc Aerob	Bac Microc	Microc B. anit

Figure 3-7. Organisms Recovered From Human Samples, Man 4

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Day	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Date	7/9/69	7/11/69	7/14/69	7/19/69	7/22/69	7/22/69	7/25/69	7/28/69	7/31/69	8/3/69	8/6/69	8/9/69	8/12/69	8/16/69	8/18/69	8/26/69
Nose	Microc	Microc Bac β-Strep	Microc Bac	Microc	Microc	Aerob	Aerob	Aerob	Bac	Aerob	Aerob	Aerob	Aerob	α-Strep	Microc	Aerob
Throat	Neisseria α-Strep	Microc α-Strep	α-Strep β-Strep E. coli	α-Strep	NGOT	NGOT	NGOT	NGOT	NGOT	NGOT	NGOT	NGOT	Aerob	β-Strep α-Strep	α-Strep	α-Strep
Rt. Ear	NGOT	Candida Prot	Prot	Prot Candida	Candida	Microc	Prot	B. anit Candida	Pseudo	Prot	Aerob	Aerob	Candida	Microc	Microc Candida	Prot Candida
Lt. Ear	Microc Bac	NGOT	Microc Candida Bac											Microc Candida	Microc	Microc Altern
Rt. Foot	Bac Candida	Microc	Microc Candida	Candida Coryne	Pseudo Aerob	Microc Candida	Aerob	Aerob	Aerob Pseudo	Aerob	Aerob	Aerob	Pseudo	Microc	Microc Altern	Microc
Lt. Foot	Microc Coryne Bac	Bac	Aerob											Prot Aerob	Microc Tricho	Microc Candida
Forehead	Mucor	Bac	Microc	Asperg	Aerob	Aerob	Microc	Microc	Microc Asperg	Microc	Aerob Microc Asperg	Aerob B. anit	Microc Asperg	Microc	Microc	Microc
Back of Neck	Microc Bac	Bac	Microc Bac Sarc											NGOT	Microc	Microc
Rt. Axilla	Microc	NGOT	Microc Bac	Bac Aerob	Bac Sarc	Microc Sarc	Microc	Aerob Sarc Bac	Sarc	Microc	Microc	Aerob	Microc Bac	Microc	Aerob Microc	Aerob Microc
Lt. Axilla	Microc Sarc	NGOT	Microc											NGOT	Microc	Microc
Rt. Groin	Microc	NGOT	Microc Sarc	Aerob	Microc	Microc	Microc α-Strep	Microc Bac	Microc	Microc Pseudo	Microc Coryne	Microc	Microc Asperg	Microc	Microc	Microc
Lt. Groin	Sarc Bac	NGOT	Microc Sarc											Microc Coryne	Microc	Microc

Figure 3-8. Organisms Recovered From Human Samples, Man 5

ORGANISMS RECOVERED FROM HUMAN SAMPLES

Mission Day	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+12
Date	7/9/69	7/11/69	7/14/69	7/16/69	7/19/69	7/22/69	7/25/69	7/28/69	7/31/69	8/3/69	8/6/69	8/9/69	8/12/69	8/16/69	8/18/69	8/26/69
Nose	Sarc	Microc	S. aureus	S. aureus	S. aureus	S. aureus	Microc	Microc Coryne	S. aureus	S. aureus Microc	S. aureus Microc	S. aureus	S. aureus	S. aureus	S. aureus	S. aureus
Throat	β-Strep α-Strep Microc Neisseria	β-Strep α-Strep	α-Strep	NGOT	α-Strep	NGOT	α-Strep	α-Strep	Candida Coryne	NGOT	α-Strep	α-Strep	S. aureus	S. aureus β-Strep	S. aureus β-Strep	Bac
Rt. Ear	Pseudo	Microc	Pseudo	NGOT	NGOT	Altern	Microc	Microc	S. aureus	S. aureus	S. aureus	Microc	Microc	Microc	Microc Altern	Microc Altern
Lt. Ear	Microc Coryne	Microc	Microc Pseudo Coryne Mold											Microc	Microc	Microc Altern
Rt. Foot	Bac Rhodo	Microc	Prot Yeast	Prot	Prot	Prot Rhodo Altern	Prot	Aerob	Prot	Prot	Prot	α-Strep	Microc	Microc Asperg	Microc Asperg	Microc
Lt. Foot	Bac Microc Mold	Microc	Prot Microc Mold											Microc	α-Strep Microc	Asperg Microc
Forehead	Bac Microc	Bac	NGOT	Microc Bac	Microc	S. aureus	Microc	Bac Aerob Microb	Microc	Microc Coryne	S. aureus	S. aureus	Microc	Coryne	Microc Bac	Microc Bac
Back of Neck	Microc	Microc Bac	NGOT											Microc	Microc	Microc
Rt. Axilla	Microc S. aureus	NGOT	Microc S. aureus	Microc	S. aureus	Microc	Aerob	Microc	Microc	Aerob	Microc	Microc	Microc	Microc Coryne	Microc	Microc
Lt. Axilla	Microc Sarc Bac	Microc Sarc	Microc Sarc Bac											Microc	Microc	Microc
Rt. Groin	Microc Sarc Bac	Microc Sarc	Microc Sarc Bac	S. aureus Microc	Sarc	Microc	Bac Aerob	Microc	Coryne	Aerob	Pseudo	Microc	Microc	Microc Bac Coryne	Microc	Microc
Lt. Groin	Microc Sarc Bac	Microc Sarc Coryne												Coryne Microc		

Figure 3-9. Organisms Recovered From Human Samples, Man 6

- Changes in immune status and response of the individual
- Difficulty in re-establishing the original flora
- Emergence of a previously controlled pathogen
- Upset of the balance in a closed ecology (bioregenerative, sanitizing systems).

Data presented in Figures 3-10 through 3-13 indicate one of the more outstanding results was a shift in the bacterial populations from the more usual gram positive organisms (Ref. 10) towards gram negative bacteria, particularly Pseudomonas and Aerobacter. The earlier studies using chambers (Ref. 3, 5, 11, 12) do not indicate the same phenomenon.

Of the differences in procedure between the GSDM and the other tests which might account for this, several are immediately apparent:

- Greater degree of ecological closure and stress
- Routine use of antimicrobial agents in soap, garments, and boat washing solutions
- Low ambient incubation temperature and long storage of mission samples.

It is common for antimicrobials to act more strongly and rapidly on gram positive organisms. This selective inhibition of one segment of the population can afford a survival advantage to those less susceptible (i. e. , gram negatives) leading to their enhanced multiplication and overgrowth.

In fact, Ehrenkranz et al (Ref. 13) as quoted by Marples (Ref. 14) reported that the intensive use of antibacterial soap on one foot suppressed the gram-positive species permitting colonization with enterobacteria. Pseudomonas was then able to colonize the skin, producing lesions which later became infected with Candida albicans.

The apparent shift towards gram negatives was undoubtedly influenced by the low incubation temperatures and long holding time in transport medium. This mitigated in favor of the hardier gram negative rods, especially those such as Pseudomonas which have lower optimum growth temperatures than most of the indigenous human flora. The method of analyzing the data, using the absolute presence or absence of an organism

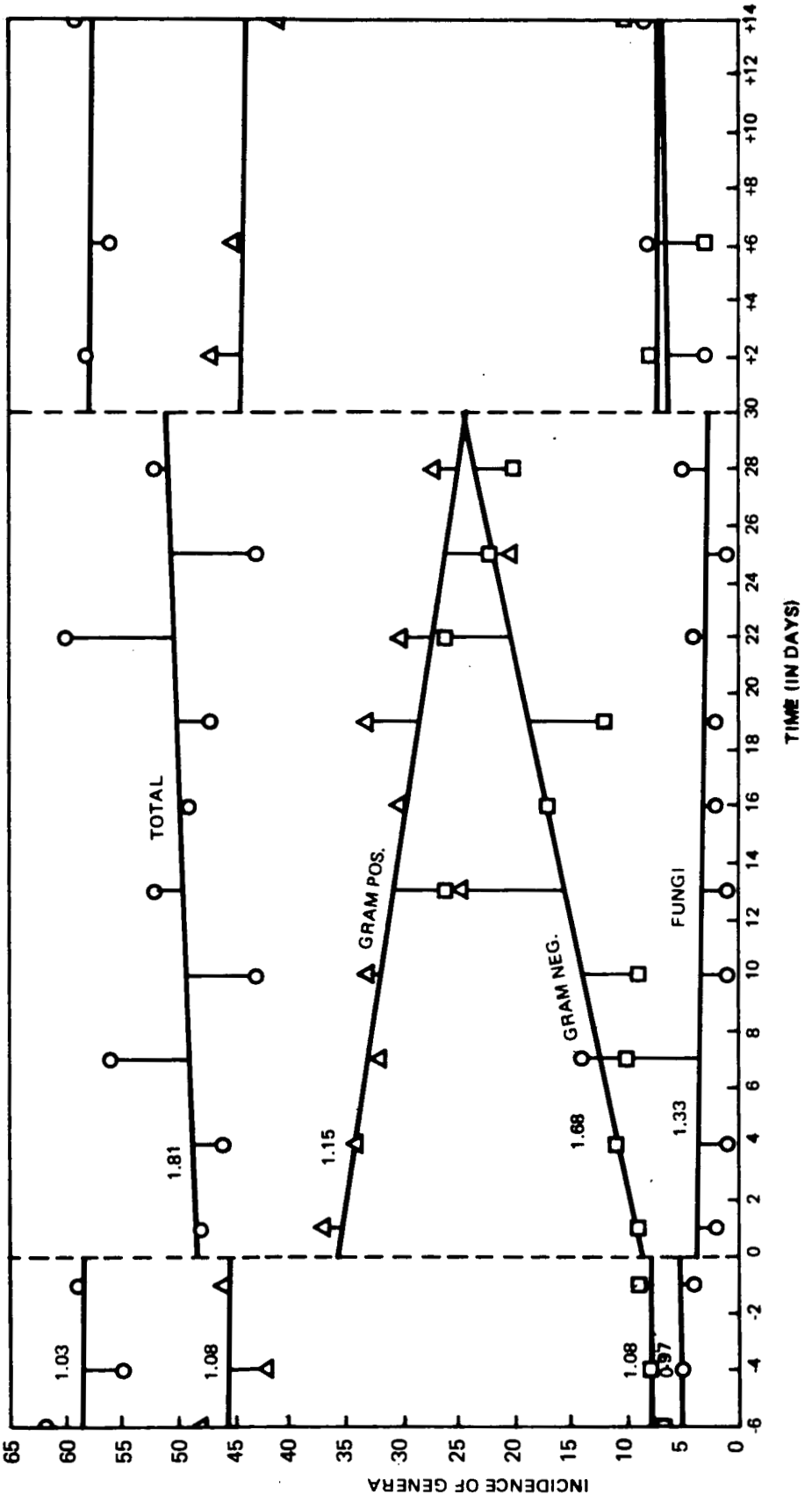

Figure 3-10. Shift: Total Body

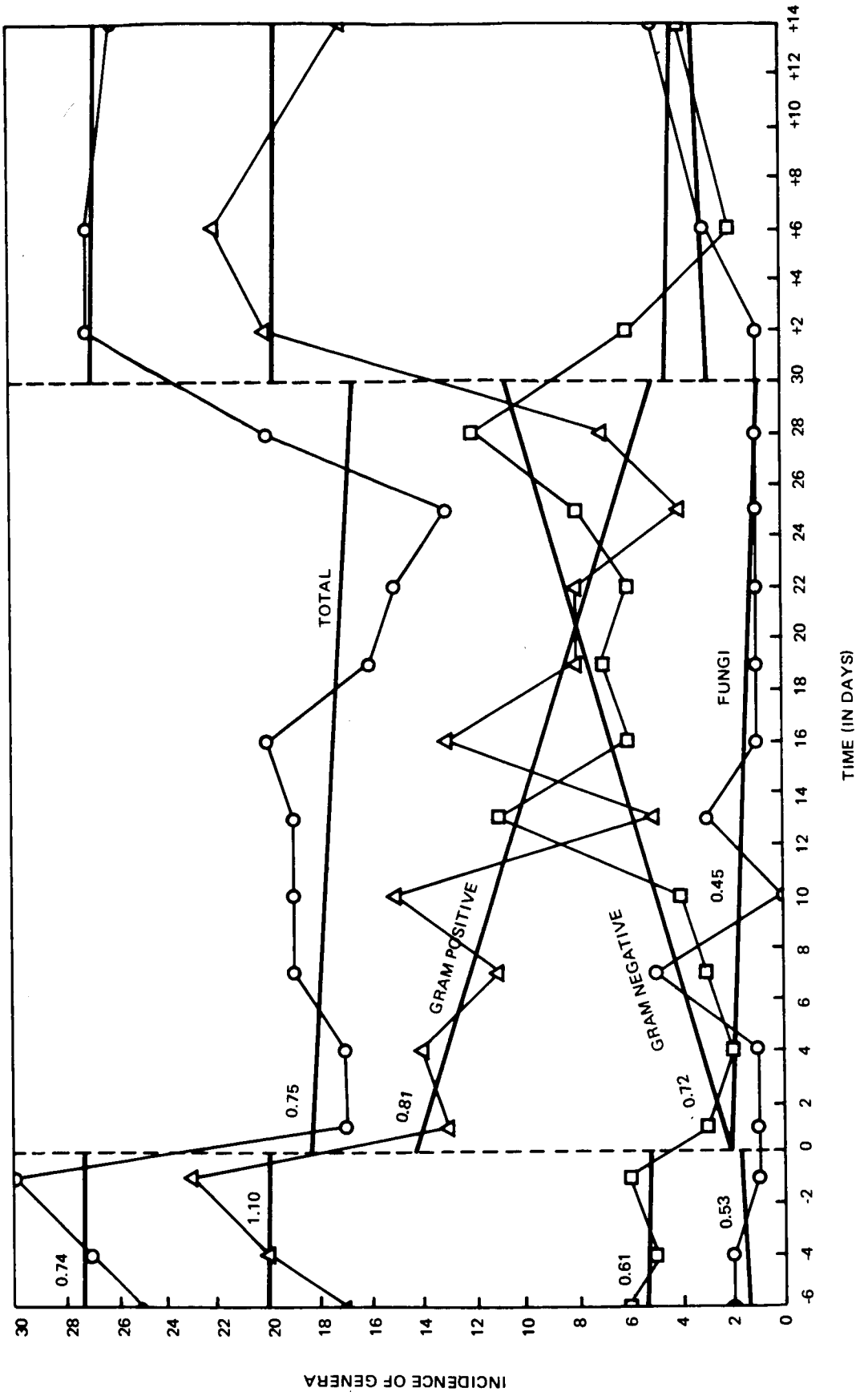

Figure 3-11. Shift: Nose, Throat & Ear

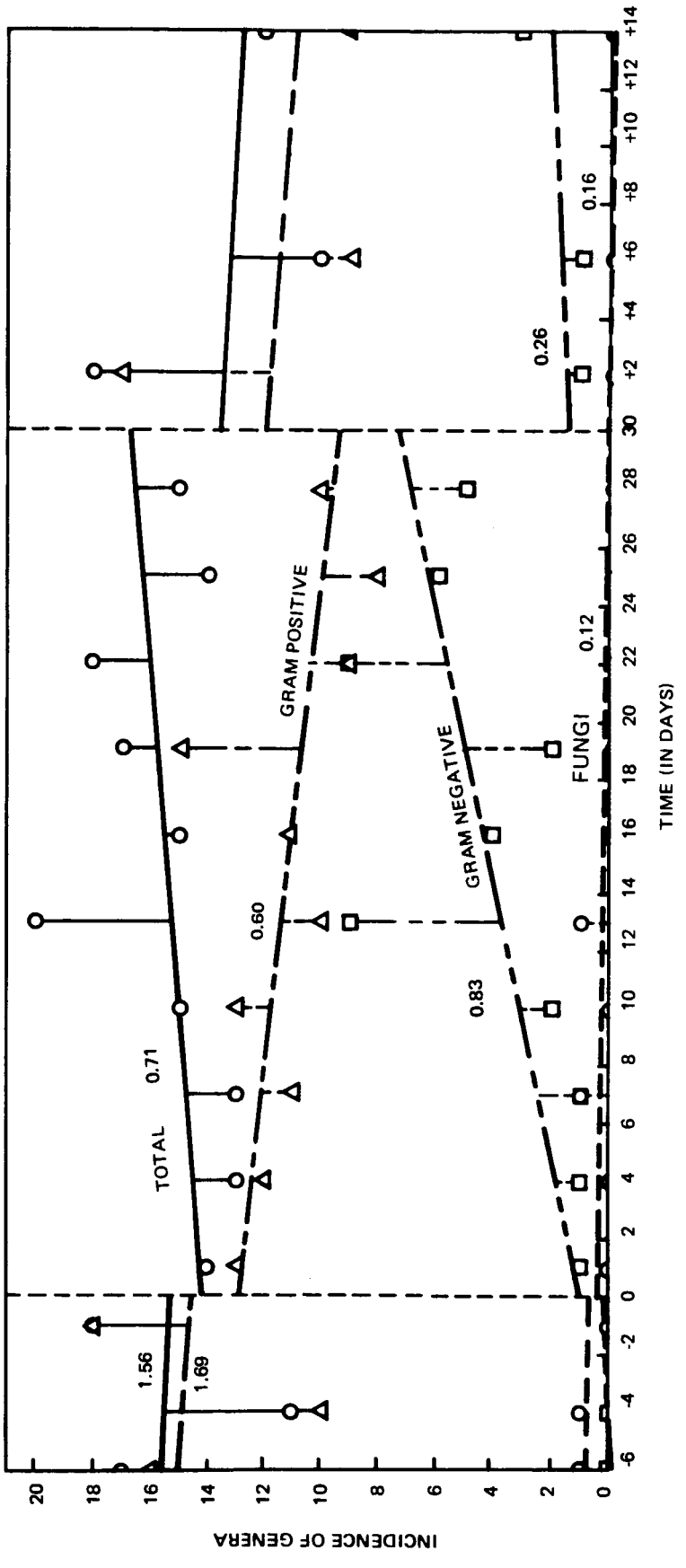

Figure 3-12. Shift: Right Armpit & Right Groin

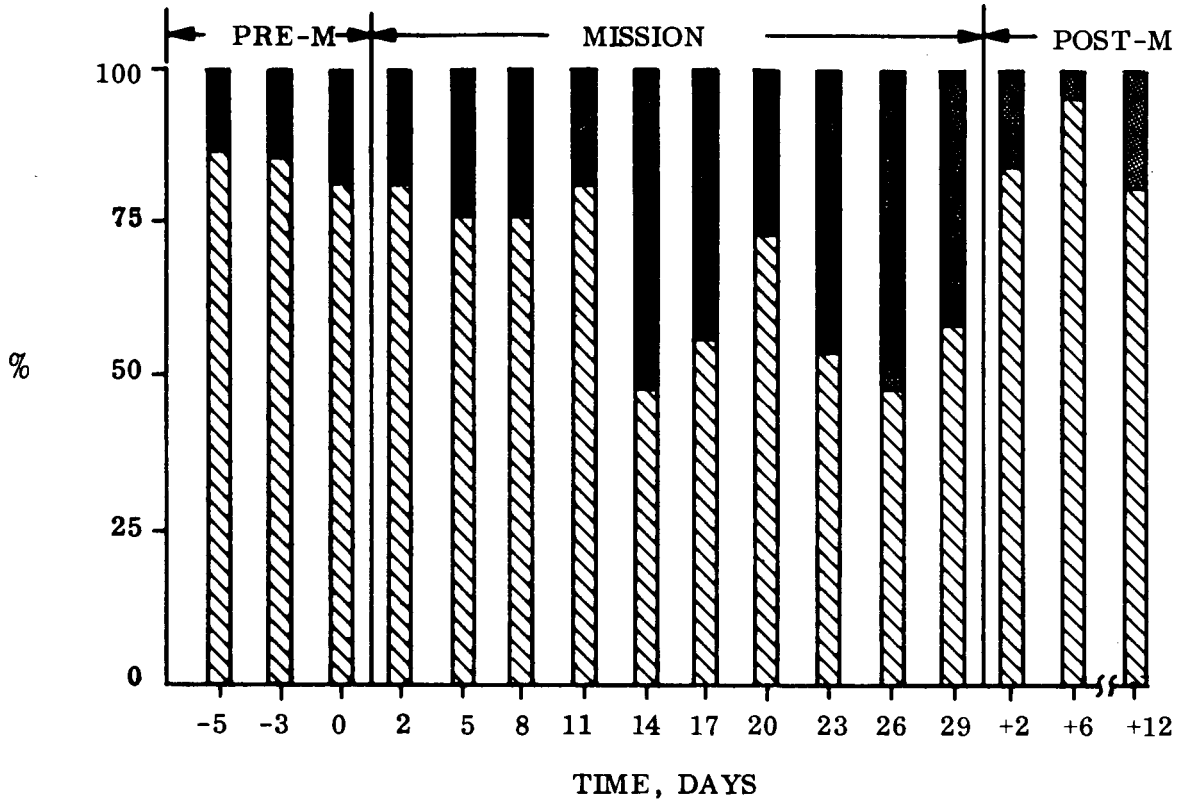

Figure 3-13. Disturbance in Human Flora
% of Occurences of Gram Pos ▨ vs Neg ■
Bacteria -6 Men, 7 Body Locations

(rather than relative numbers in each sample), was not desirable but was the only approach possible under the test conditions.

Another major observation was a general simplification of flora (Figures 3-14 and 3-15) as evidenced by a decreased number of different genera isolated as the mission progressed. This has been postulated by Luckey (Ref. 15) as an effect of biological isolation, but had not previously been demonstrated in manned chamber operations. With a continued simplification of flora there is a greater potential for lowered resistance, the emergence of opportunistic pathogens and sudden shock upon return to a conventional environment. Conversely, during long term missions, if an isolated crew has adapted to live in symbiosis with a high level of "abnormal" bacteria, health problems could arise in the fresh crew members upon resupply or crew exchange. This is akin to the Staph carrier who, while not clinically ill himself, can infect and cause disease in others. The evidence for microbic shock has been demonstrated in the laboratory using germ free and isolated animals and there is also a parallel to the common phenomenon in healthy individuals travelling over great distance in short periods of time. The incidence of respiratory infection in new arrivals is quite high when contact is made with a somewhat different set of indigenous microbes.

So far as the data from this mission are concerned, there were still gram positive organisms remaining at the end of the mission and the men remained healthy. However, health problems could arise if fresh crew members were introduced or on resupply or new exchange situations.

This simplification occurred overall, although the curve was steeper for the body taken as a whole than for nose, throat, and ear which were less affected by germicides. It may, therefore, be to some degree independent of germicide action except for consideration of which organisms will survive during the simplification process. (This relates back to the questions and problems in sampling raised in Subsection 2.4). The greater rate of simplification for the body, taken as a whole, as opposed to nose throat and ear where germicide action is minimal, emphasizes the influence of antimicrobials on the simplification process.

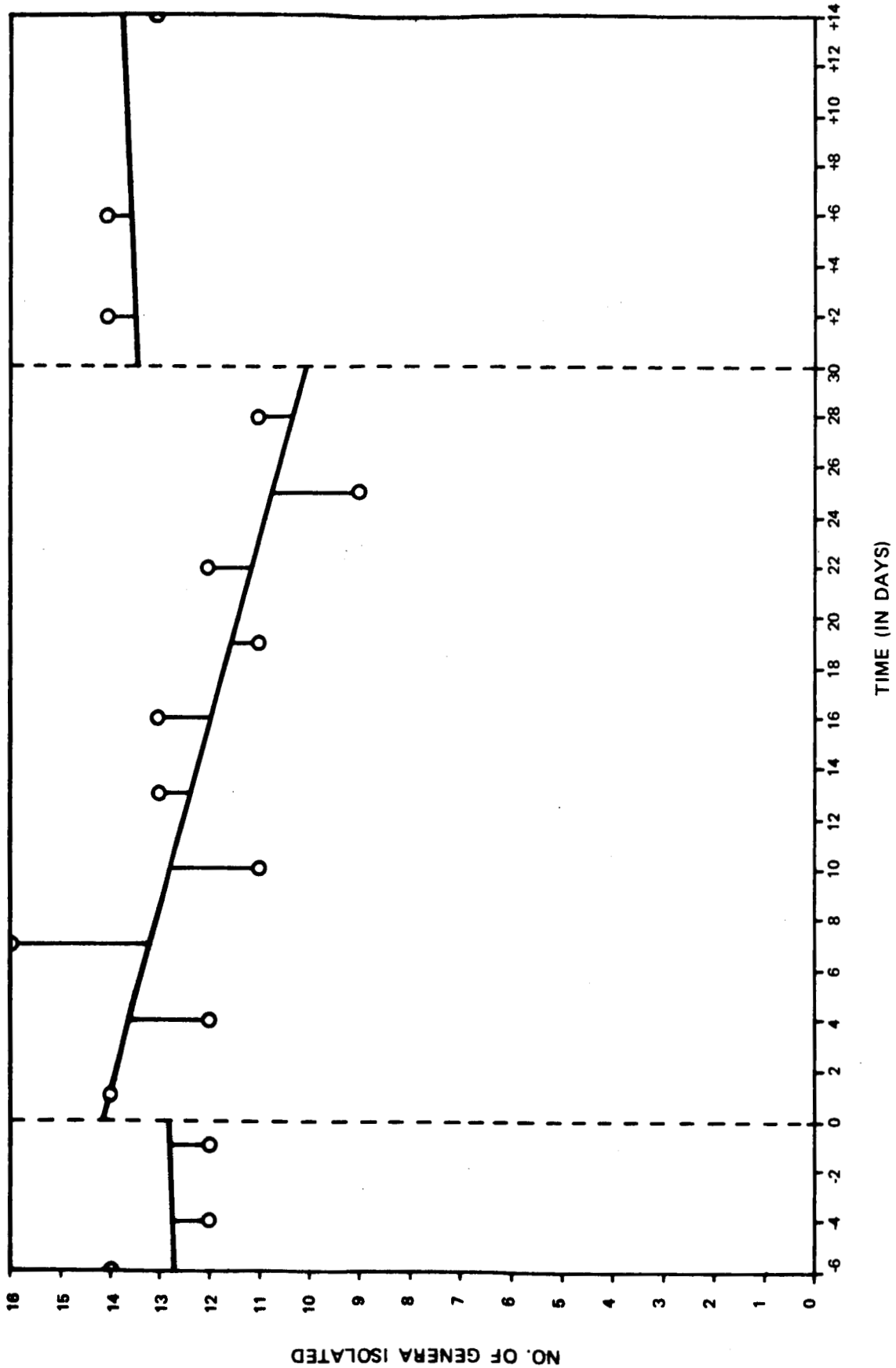

Figure 3-14. Simplification: Total Body

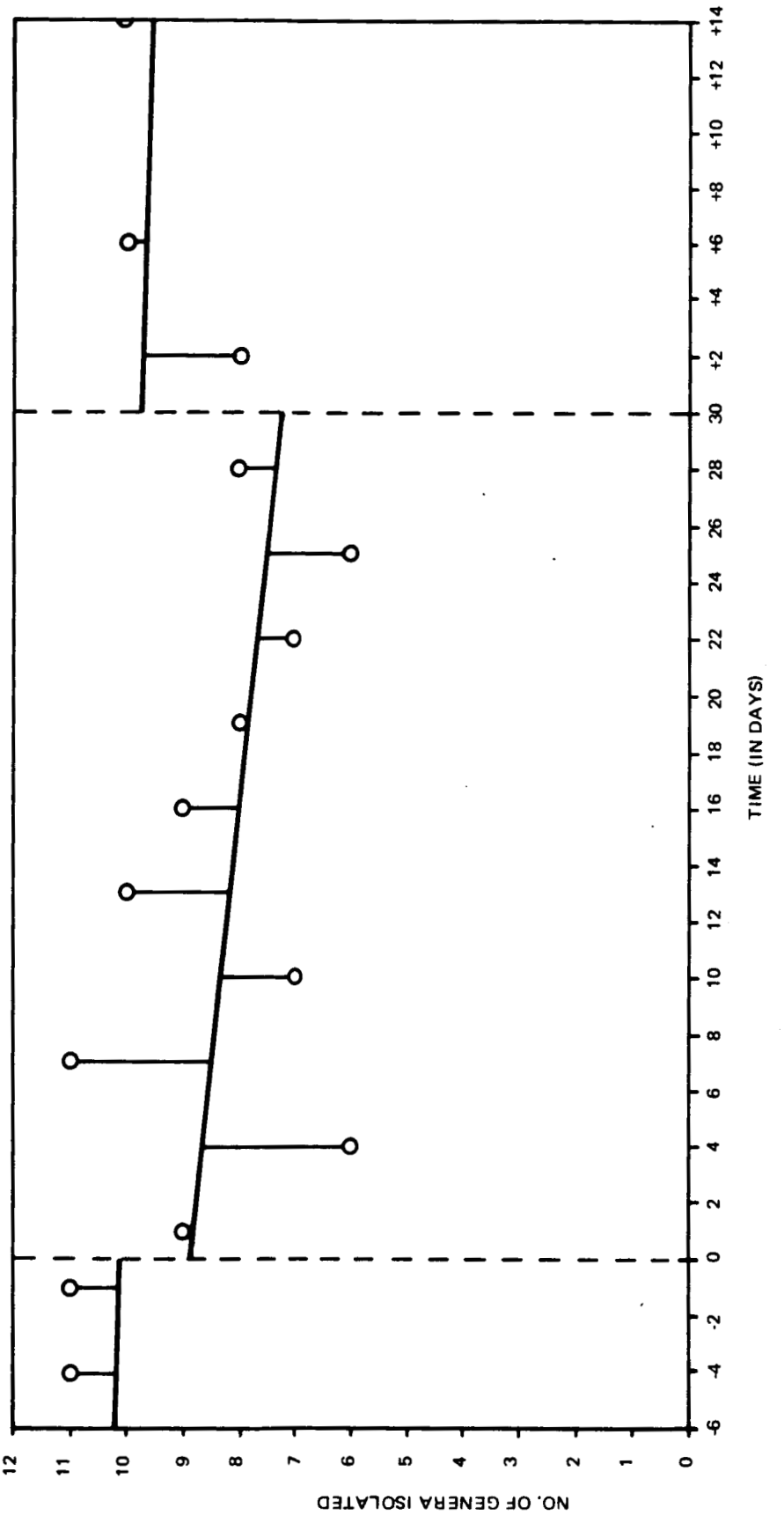

Figure 3-15. Simplification: Nose, Throat & Ear

Regardless of the operative mechanisms, if this unbalancing of the body's ecology truly occurs, it deserves serious consideration in the planning of future long-term space missions and a re-evaluation of the use and selection of antimicrobial agents. On longer missions one might consider deliberately introducing gram positive forms into the environment if the balance became seriously upset.

3.1.3 Specific Bacteria

Several organisms are of special interest due to their potential pathogenicity and/or proliferation and are discussed individually below.

3.1.3.1 Staph aureus

Beta hemolytic Staph aureus was easily followed throughout this study (Figures 3-16, 3-17). Although Crewman 6 had a history of minor nose infections, Staph aureus was not detected during the pre-mission period, either in his samples or in other crew members. It did, however, occur on the first sampling period of the mission, after 2 days of biological isolation, and persisted in samples from Man 6 throughout the mission and post-mission.

It cannot be unequivocably stated whether the appearances of Staph aureus on Men 1, 2, and 4 indicate a transfer from Man 6 since no phage typing was done, but it is obvious that these invasions were transient in nature and in no case did permanent colonization occur on Men 1, 2, and 4. At no time was Staph aureus recovered from Men 3 or 5, indicating a lack of invasiveness.

Results from other chamber studies (Ref. 1, 12) also indicate an upsurge of Staph aureus. In the Douglas test, Staph aureus was isolated from 3 of the 4 subjects (1, 3, and 4) before the start of the mission and continued to appear throughout and after completion of the test. However, significantly, it never transferred to their Subject 2 and "no problems attributable to Staphlococci occurred during the test and these subjects emerged after 60 days still carrying the organism" (Ref. 1).

In the ACEL test (Ref. 12) a variety of Staph aureus cultures were isolated and phage typed. It is interesting to note that once again transferrence of the Staph

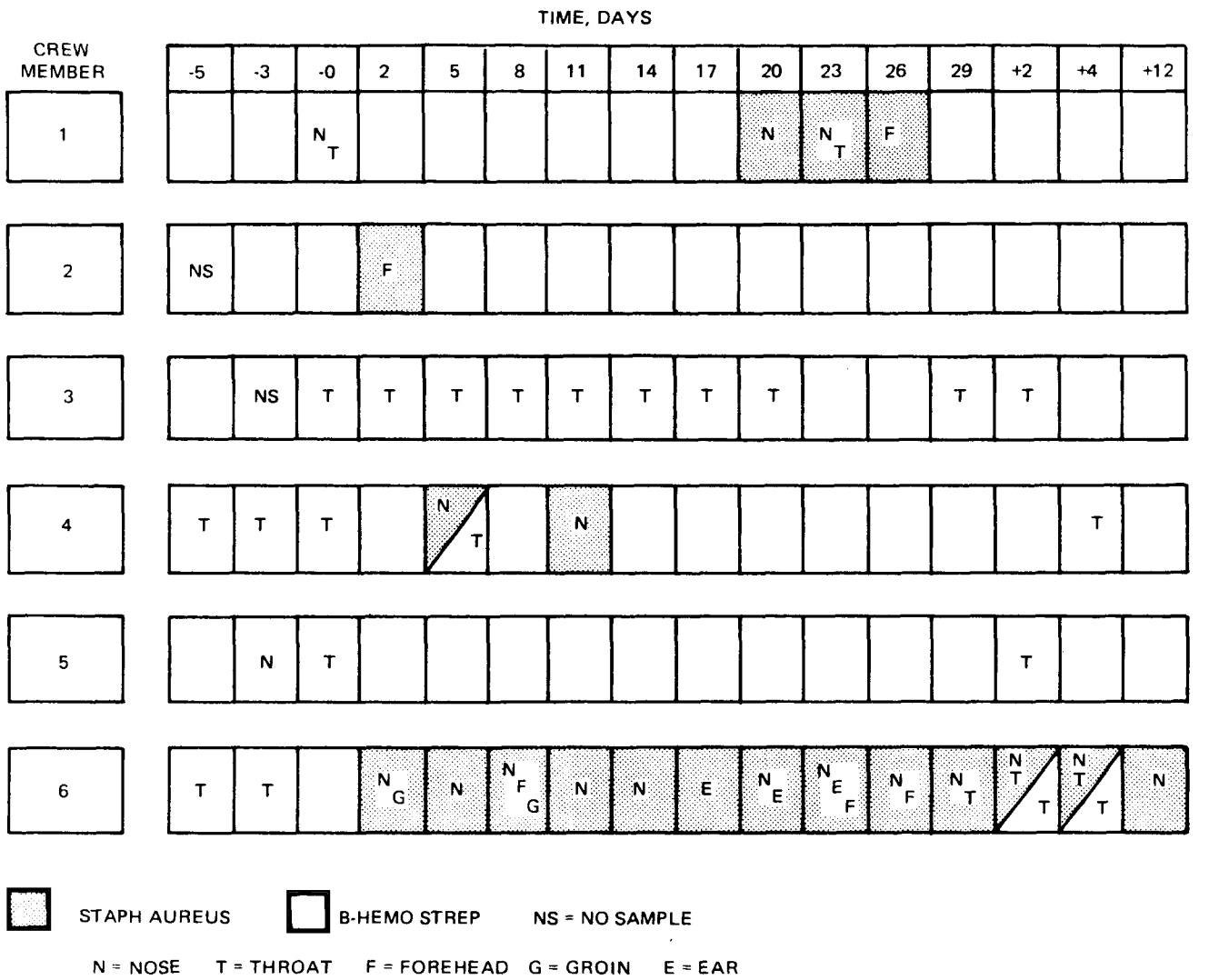

Figure 3-16. Incidence of Potential Pathogens

STAPH AUREUS

	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+8	+12
HUMAN				2-FH 6-RG N	4-N 6-N	6-N 6-FH 6-RA	4-N 6-N	6-N	6-RE	1-N 6-N RE	1-N 1-THR 6-N FH RE	1-FH 6-N FH	6-H THR	6-N THR	6-N THR	No Sampling	6-N
ENVIRONMENT				HS GB SF	AHF GF			WR TT GS FHF									

PSEUDOMONAS

HUMAN	1-N THR LF 2-LF 6-RF	2-RE	1-THR 2-LF 6-LE RE RF	1-THR 4-RF	2-RF 5-RF 6-RG	1-THR	3-RF	1-THR 3-N	1-RG 5-RF RE 4-RE	3-RA 4-THR 5-RG	4-FH RG	3-RA RE 6-RG	1-RA 3-FH RE	4-N			2-LF
ENVIRONMENT	HF(-7)				Air	GS FHWP	SF GF Air	SF FHWS	TT SW WB FHWP	GF * AHF SW SF WB	GF * GS GB AHF Air	SW * WB GS FHF AHF				SW SS HS GF AHF AHB	
WATER			GS1, 2, 3, 4 HS SS	GS SS	GS SS	HSF GS SS		HS	GS HS SS	GS	HS SS	HS SS				GS Trap, Filter HS Trap, Filter Toilet	
MISC		•				3-Sheet Top 3-Sock		4-T Shirt	5-Pillowcase							Sponge 5-Washcloth	

LEGEND:

HUMAN

BN	=	Back of Neck	AHF	=	Aft Hemi Floor
FH	=	Forehead	FHF	=	Forward Hemi Floor
N	=	Nose	FHWP	=	Forward Hemi Wall Port
LA	=	Left Axilla	FHWS	=	Forward Hemi Wall Starboard
LE	=	Left Ear	GB	=	Garage Bin
LF	=	Left Foot	GF	=	Galley Floor
LG	=	Left Groin	HF	=	Head Floor
RA	=	Right Axilla	SF	=	Shower Floor
RE	=	Right Ear	SW	=	Shower Wall
RF	=	Right Foot	TT	=	Table Top
RG	=	Right Groin	WB	=	Work Bench
THR	=	Throat	WR	=	Ward Room

ENVIRONMENT

WATER

GS	=	Galley Sink
HS	=	Head Sink
HSF	=	Head Sink Filter
SS	=	Shower Sink

▲ = Boat Scrubbed

*NOTE: Environment sampled days 21, 24, 27 not 20, 23, 26

Figure 3-17. Occurrence of Specific Bacteria (Sheet 1 of 3)

BACTERIUM ANITRATUM

	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+8	+12
HUMAN						4-RA	4-RA	1-FH 2-RG 4-RG 5-RE	1-FH RA 3-RA	1-RA	2-RF	5-FH	2-RA 2-RG 4-RG	4-LA	2-LE 2-BN 4-FH RA LA		1-FH 4-RE
ENVIRONMENT					Air		AHF	TT WB FHF HF GB	TT	TT AHW	FHF WB *	GB *	GB *			GB WRS	
WATER								SS								6-Blanket	

▲ ▲

PROTEUS

	4-RF	1-THR 4-RF 5-RE	2-LF 4-RF 5-RE	4-RF 5-RE	2-RF 3-RF 4-RF 6-RF LF	2-RF 3-RF 6-RF	4-RF 5-RE 6-RF	3-RF 4-RF 6-RF		4-RF 5-RE 6-RF	4-RF 6-RF	4-RF 6-RF		4-RF LF	4-LF		4-LF 4-RF 5-RE
HUMAN																	
ENVIRONMENT					SF			SF	SF HF Air		SF *	SF *					
WATER									HS								
MISC																Bilge Main Waste Tank	Shower Filter

▲ ▲

LEGEND:

HUMAN

BN	=	Back of Neck
FH	=	Forehead
N	=	Nose
LA	=	Left Axilla
LE	=	Left Ear
LF	=	Left Foot
LG	=	Left Groin
RA	=	Right Axilla
RE	=	Right Ear
RF	=	Right Foot
RG	=	Right Groin
THR	=	Throat

ENVIRONMENT

AHF	=	Aft Hemi Floor
FHF	=	Forward Hemi Floor
FHWP	=	Forward Hemi Wall Port
FHWS	=	Forward Hemi Wall Starboard
GB	=	Garage Bin
GF	=	Galley Floor
HF	=	Head Floor
SF	=	Shower Floor
SW	=	Shower Wall
TT	=	Table Top
WB	=	Work Bench
WR	=	Ward Room

WATER

GS	=	Galley Sink
HS	=	Head Sink
HSF	=	Head Sink Filter
SS	=	Shower Sink

▲ = Boat Scrubbed

*NOTE: Environment sampled days 21, 24, 27 not 20, 23, 26

Figure 3-17. Occurrence of Specific Bacteria (Sheet 2 of 3)

OCCURRENCE OF AEROBACTER

	-5	-3	0	2	5	8	11	14	17	20	23	26	29	+2	+4	+8	+12
HUMAN		1-THR 2-RF	2-RE LE RF 4-THR 5-LF	5-RA RG	5-RF FH	2-FH 5-N FH	1-RE RA 2-FH 3-RE RF 5-N RF	1-RE, RF, FH 4-N, FH, RA, RG 6-RF, RA, RG 2-N, THR, RE, RG 3-RE, RA, FH 5-N, RF, RA	2-RF 3-N, RE 4-THR, RG 5-RF	2-RE 3-RE 4-N 5-N, RF	1-RF, RA, RF 5-N, RE, RF, FH 2-RE, RG 3-N, RE, RA, FH 4-N, FH 6-RA, RG	1-THR 2-N, RE, FH, RG 3-N, THR, RA 4-N, THR, RE, FH 5-N, RE, RF, FH, RA	1-THR 2-N, THR, RE, RF, RA 3-N, RE 4-N, RE, RF 5-N, THR	2-RE, RF RH 4-RG 5-LF	2-RE 5-RA		1-LF RA 2-RE, LE 5-N, RA
ENVIRONMENT					Air		SW	SW GF WB	SF	SW * SF Air	SW * SF	FHF * GF SF				GF Air	
WATER						HS SS	GS HS	HS		SS			GS			SST	
MISC						4-T Shirt										3-T-Shirt 3-Jumpsuit 3-Sheet 5-Blanket 5-Washcloth	

▲ ▲

LEGEND:

HUMAN

BN	=	Back of Neck
FH	=	Forehead
N	=	Nose
LA	=	Left Axilla
LE	=	Left Ear
LF	=	Left Foot
LG	=	Left Groin
RA	=	Right Axilla
RE	=	Right Ear
RF	=	Right Foot
RG	=	Right Groin
THR	=	Throat

ENVIRONMENT

AHF	=	Aft Hemi Floor
FHF	=	Forward Hemi Floor
FHWP	=	Forward Hemi Wall Port
FHWS	=	Forward Hemi Wall Starboard
GB	=	Garage Bin
GF	=	Galley Floor
HF	=	Head Floor
SF	=	Shower Floor
SW	=	Shower Wall
TT	=	Table Top
WB	=	Work Bench
WR	=	Ward Room

WATER

GS	=	Galley Sink
HS	=	Head Sink
HSF	=	Head Sink Filter
SS	=	Shower Sink

▲ = Boat Scrubbed

*NOTE: Environment sampled days 21, 24, 27, not 20, 23, 26

Figure 3-17. Occurrence of Specific Bacteria (Sheet 3 of 3)

aureus was not demonstrated even though some Staph spread to the environment; and there were no instances of overt illness attributable to Staph aureus.

These results seem to be in basic agreement with data from the GSDM. Even so, in considerations of long term missions, the fate and transmission of these organisms are of great importance because of their pathogenic nature, particularly in lowered resistance states, and where the skins normal integrity has been damaged either through abrasion, sweating, chemicals, or burns, etc.

3.1.3.2 Beta Hemolytic Streptococci

Prior to the start of the mission, Beta hemolytic Streptococci were isolated from 5 of the 6 crew members. (Figure 3-16) The same men reported minor upper respiratory infections (URI) after the start of the mission. The remaining crew member, Man 2, had neither the Strep nor the URI. He also appeared to have the most firmly establishing alpha Strep population which could have inhibited invasion by the beta Strep.

It is possible to speculate that while the 5 carriers of beta Strep were able to stay healthy in their normal environment, the change in environment and attendant stress upon entering biological isolation caused an inbalance sufficient to produce URI symptoms. Once they adapted to the new environment, the symptoms disappeared.

3.1.3.3 Bacterium anitratum

Bacterium anitratum first appeared about 5 days into the mission (Figure 3-17) when it was isolated from the air, although the original source is suspected as having been human. Its most widespread recovery was on Day 14 of the mission, but from Day 5 on, it was recovered from the men, the environment or both at each subsequent sampling period. This unusual organism has previously not been recovered in manned chamber operations. Its emergence and persistence is noteworthy since recent studies indicate that not only is it a part of the normal skin flora, but is implicated as an opportunistic pathogen in burn, wound and urinary tract infections and pneumonias. Its competitive relationship with other bacteria has not been studied, but this organism is generally known to be antibiotic resistant.

3.1.3.4 Pseudomonas

This organism was the most universal contaminant recovered, being isolated from the water, the men, and the environment (Figure 3-17) with great frequency. It was isolated pre-mission from some of the crewmen. During the mission it spread throughout the crew. It was present in the water (based on post-mission analysis of mission samples) from Day 1 of the mission although it was not detected by on-board monitoring until Day 8. The spread of Pseudomonas undoubtedly was enhanced by its presence in the water. In the Douglas chamber test, no Pseudomonas was found on the subjects despite contamination of their water system with this organism. However, their test did not include the use of antimicrobials which suppressed the competitive microbes.

The increasingly frequent occurrence of Pseudomonas in clinical infections, (to the point where they are replacing Staph aureus as the organism of greatest concern in hospital infections), and their resistance to many antibacterial agents makes their widespread recovery on the GSDM and its crew a cause for concern in case of accidents, etc. Measures to control this organism should be investigated.

3.1.3.5 Proteus

This is a highly pathogenic organism when found outside of the GI tract. It often acts as a secondary invader in dermatitis, particularly of the feet, and is noted for its resistance to eradication. It can be noted that the original isolation was from a crew member's feet (Figure 3-17) after which it spread to the shower floor and to other crewmen. As with Aerobacter, the foremost reservoir was the shower floor.

One cause of concern during the laboratory work-up of the microbiology samples was the lack of recovery of certain fastidious organisms, such as Neisseria in the throat; and of the anerobes and corynebacteria which, although delicate to culture, are important segments of the major true or indigenous skin flora. Some of this is undoubtedly due to culturing constraints such as transportation, storage and incubation temperatures which would mitigate against their survival. However, their complete absence during the mission, implies some effect other than simply culture technique.

Figure 3-18 presents a tabulation and summary of the human sampling.

BEN FRANKLIN HUMAN FLORA SAMPLES

	Pre-Mission	Mission	Post-Mission	Total
Total Samples Taken For				
Microbiology	199	418	214	831
Chemical Analysis	0	0	0	0
Total Number of Isolates	401	522	291	1214
Total Colonies Picked	401	943	367	1711
Total Number of Sterile Samples	0	0	0	0
On Board Readings	N/A	51	N/A	51
Laboratory Readings	0	1	0	1
Total Samples Lost	22	16	3	41
In Shipment	0	0	0	0
In Storage Too Long - (NGOT)	22	16	3	41
Total Number of Isolates Identified to Genus	391	522	291	1711
Total Number of Genera Found	15	16	16	21

Figure 3-18. Summary: Ben Franklin Human Flora Samples

3.1.4 Conclusions

- Man is capable of existing with no serious illness in biological isolation for a 30-day period with a simple life support system under the conditions found in the GSDM
- Data reliability was affected by low-temperature incubation, long storage, and antimicrobials
- Flora became unbalanced: simplification occurred; shifts towards gram negative rods occurred; final limits of shift and simplification were not reached in 30 days
- Even in the common environment, individual differences persisted among the crew
- Use of antimicrobials may influence direction of shift
- While some advantages may accrue from antimicrobial treatment, the overall effect may be undesirable.

3.1.5 Recommendations

- Formulate an experimental design to more accurately define shifts and simplification of flora
- Perform more stringent testing of antimicrobial treatment versus non-treatment: - control subjects during mission - establish comparable baseline data prior to and after the mission under controlled conditions - study various antimicrobials particularly with regard to selective inhibition
- Refine the on-board monitoring system with the possibility of on-board facilities to assure maintenance of sanitation standards or signal deterioration so that remedial action can be instituted before catastrophic failure
- Investigate an automated monitoring system which could: - reduce the manpower required - eliminate need for trained microbiologist - provide closer to real time reporting - allow more data to be processed
- Investigate deliberate inoculation of gram positive forms.

3.2 ENVIRONMENT

Sampling of the environmental surfaces and air was accomplished according to the schedule in Figure 2-1. Pre-mission sampling was limited to two periods before loading. No samples were taken immediately after cleaning but prior to the crew's entry into the boat. A diagram of the surfaces sampled is presented in Figure 2-3.

The entire boat was thoroughly scrubbed with detergent and water on Day 0 prior to loading. During the mission, the surfaces such as table, bench and sink tops were wiped daily with a sanitizing agent. On Days 7, 14, and 21 there was a general cleaning of the boat, using the quaternary amine sanitizer. On Day 21 the galley, head and shower floors were washed thoroughly with the quaternary amine solution and were washed daily thereafter. Towards the end of the mission a general laxity in the cleaning procedures was reported.

3.2.1 Total Counts

Figure 3-19 presents the total counts for each area sampled, grouped as to floors, walls, and surfaces. These data are also displayed in Figure 3-20. The initial clean-up appeared to have lowered the microbial load considerably, since at Day 2 the counts were all quite low. The dips in contamination levels can be related to cleaning procedures with transient drops in microbial counts on the walls and floors noted at the general clean-ups. However, a rapid rise on the walls and floors followed cleaning, and a generally upward trend was observed. With respect to the table surfaces, the picture is somewhat different. This can be attributed to the daily washing protocol which resulted in a much slower rise in total populations. The above results contrast with other studies (Ref. 3) where, after initial disinfection, no cleaning procedures were instituted and a level of contamination was reached which remained relatively constant throughout the mission. This plateau phenomonon has also been observed in a study of clean rooms and conventional manufacturing areas. It is considered to be a dynamic rather than static situation in which "the number of microorganisms deposited on or surviving on surfaces is balanced by the number of microorganisms dying on the same surface" --- "such factors as the absence of nutrients, humidity, temperature and types of microorganisms influence the survival rate on surfaces." (Ref. 16). Diversions from this plateau effect may be attributed to the

ENVIRONMENTAL SURFACES MICRO-ORGANISMS/4 IN^2

(LETHEEN AGAR RODAC PLATE)

Mission Day	-7	-2	2	5	8	11	14	17	21	24	27	Mission Avg		+8
Fwd Hem Floor	NS	TNTC	100	70	$NC_{(M)}$	701	43	95	40	TNTC	70	173		TNTC
Aft Hem Floor	TNTC	NS	$NC_{(M)}$	300	50	180	85	TNTC	TNTC	TNTC	84	462		72
Galley Floor	TNTC	TNTC	5	55	2	TNTC	110	250	250	TNTC	NC	408		TNTC
Shower Floor	TNTC	TNTC	20	200	NC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	691		$NC_{(S)}$
Head Floor	TNTC	TNTC	$NC_{(M)}$	54	112	150	TNTC	TNTC	170	110	$NC_{(M)}$	338		66
Floor Avg	1000	1000	42	139	87	92	448	669	492	822	453	420		535
Fwd Hem Wall Port	128	TNTC	NS	5	75	50	1	1	4	55	0	24		0
Fwd Hem Wall Stbd	7	63	1	3	0	0	$NC_{(S)}$	1	$NC_{(S)}$	194	11	23		42
Aft Hem Wall	1	TNTC	$NC_{(M)}$	300	80	4	$NC_{(M)}$	22	28	0	0	55		0
Shower Wall	4	TNTC	NS	7	$NC_{(M)}$	TNTC	200	TNTC	90	19	TNTC	44		2
Wall Avg	35	166	1	78	40	263	51	256	31	67	253	126		11

LEGEND:

TNTC = Too numerous to count

NC = No count

$NC_{(M)}$ = No count - mold overgrowth

$NC_{(S)}$ = no count - spreader

NS = No sample

For Purposes of Averaging:

(1) TNTC taken as 1000

(2) NC taken as previous count with exception of Day 1

Figure 3-19. Number of Micro Organisms per 4 sq. in. on Environmental Surfaces (Sheet 1 of 2)

ENVIRONMENTAL SURFACES MICRO-ORGANISMS/4 IN^2

(LETHEEN AGAR RODAC PLATE) (Continued)

Mission Day	-7	-2	2	5	8	11	14	17	21	24	27	Mission Avg		+8
Galley Sink Formica	TNTC	TNTC	35	2	7	8	36	12	$NC_{(S)}$	40	29	20		$NC_{(M)}$
Galley Sink Stainless	TNTC	23	50	$NC_{(M)}$	$NC_{(S)}$	3	8	$NC_{(S)}$	$NC_{(S)}$	$NC_{(S)}$	17			2
Wardrm Table Top	79	7	$NC_{(M)}$	5	$NC_{(M)}$	10	33	52	50	78	21	36		8
Bench Top Port	TNTC	13	$NC_{(M)}$	15	12	42	36	80	60	95	105	56		26
Surfaces Avg	770	264	42	18	17	14	19	35	31	53	51	31		12
Head Seat	8	NS	150	NS	NS	NS	NS	NS	NS	NS	NS	150		12
Garbage Bin	7	TNTC	1	2	3	30	30	60	95	93	62	42		2

LEGEND:

TNTC = Too numerous to count

NC = No count

$NC_{(M)}$ = No count - mold overgrowth

$NC_{(S)}$ = No count - spreader

NS = No sample

For Purposes of Averaging:

(1) TNTC taken as 1000

(2) NC taken as previous count with exception of Day 1

Figure 3-19. Number of Micro Organisms per 4 sq. in. on Environmental Surfaces (Sheet 2 of 2)

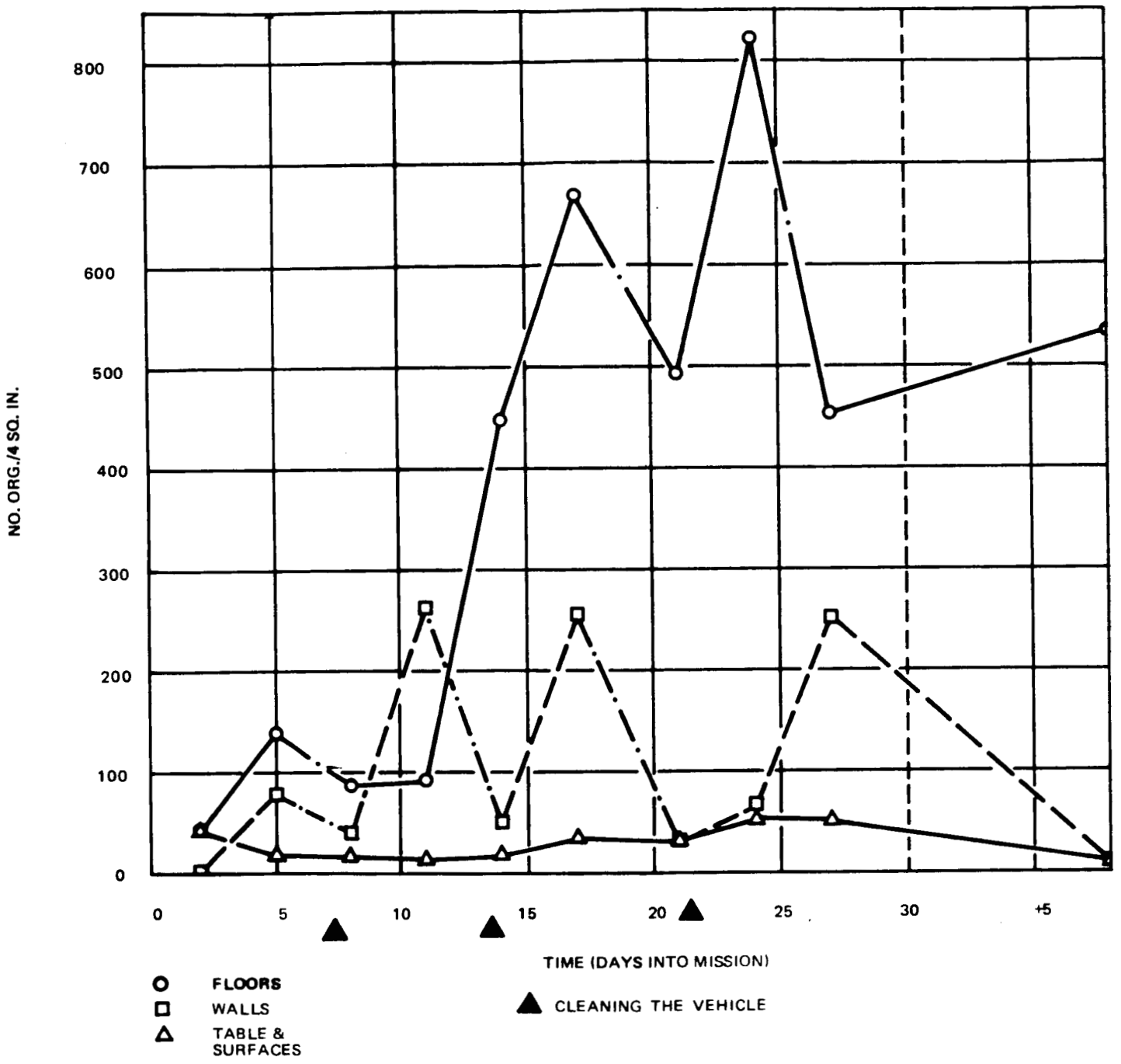

Figure 3-20. Microbial Growth vs Time

cleaning and sanitizer action. By removing the toxic end products of bacterial metabolism and by selectively killing one segment of the population, others may be given a survival advantage leading to multiplication and overgrowth. It can be noted that towards the end of the mission, when cleaning became more lax, a drop in the counts and trend towards stabilization of contamination seemed to occur.

Analyzed in a different manner, the data in Figure 2-21 indicate the personal hygiene area to have been the dirtiest part of the boat and the shower floor to be the most contaminated area. This is not surprising considering the usage of the area, the usually wet condition of the floor and the fact that crew members were in bare feet spreading their microorganisms throughout the area.

Figure 3-22 presents the results of the Andersen (air) sampling, showing on-board and post-mission base laboratory readings of the same sets of plates. As in many other instances, discrepancies exist between the two, with the base laboratory counts being generally higher. In addition, with the increased storage time, molds and "spreaders" often covered the plates making base laboratory counts impossible.

Both sets of data, however, do indicate a peak level at Day 21 which corresponds with reports of a bad odor emanating from the waste tank vent. Washing the boat and eliminating the discharge of odor from the vent was accompanied by a temporary reduction in the airborne bacterial levels.

Attempts to correlate airborne bacterial levels with surface contamination levels has met with little success and it is quite possible that the Anderson counts could be more closely tied to activity within the boat at the time the samples were taken. However, this information is not available.

3.2.2 Types of Organisms Recovered

An analysis of the types of microorganisms recovered is presented in Figures 3-22 and 3-23. The initial bacterial load consisted mainly of gram positive bacteria, but, as the mission progressed, gram negative organisms were recovered with increasing frequency. Especially noteworthy was the presence of Aerobacter and Proteus which are human associated enterics, and Pseudomonas which was virtually universal in the GSDM. From Figure 3-17, it appears that the shower floor provided an excellent reservoir for

ENVIRONMENT SURFACES - ACCORDING TO BOAT AREA

	-7	-2	2	5	8	11	14	17	21	24	27	+8	Mission Avg
Galley													
Floor	TNTC	TNTC	5	55	2	TNTC	110	250	250	TNTC	NC	TNTC	408
Sink Stainless	TNTC	23	50	$NC_{(M)}$	$NC_{(S)}$	3	8	$NC_{(S)}$	0	$NC_{(S)}$	$NC_{(S)}$	2	17
Sink Formica	TNTC	TNTC	35	2	7	8	26	12	$NC_{(S)}$	40	29	$NC_{(M)}$	20
AVG	1000	2023/3 674	90/3 30	107/3 36	519/3 201	1011/3 337	144/3 48	270/3 90	263/3 88	1040/3 345	1029/3 343	1031/3 344	115
FWD													
Floor	NS	TNTC	100	70	$NC_{(M)}$	70	43	95	40	TNTC	70	TNTC	173
Wall-PORT	128	TNTC	NS	5	75	50	1	1	4	55	0	0	24
Wall-STBD	7	63	1	3	0	0	$NC_{(S)}$	1	$NC_{(S)}$	194	11	42	23
Ward RM Table	79	17	$NC_{(M)}$	5	$NC_{(M)}$	10	33	52	50	78	21	8	36
AVG	214/3 71	2080/4 520	118/3 39	83/4 21	150/4 37	130/4 32	77/4 19	149/4 37	95/4 24	1327/4 332	102/4 26	1050/4 262	63
AFT													
Floor	TNTC	NS	$NC_{(M)}$	300	50	180	85	TNTC	TNTC	TNTC	84	72	462
Wall	1	TNTC	$NC_{(M)}$	300	80	4	$NC_{(M)}$	22	28	0	0	0	55
Bench Top	TNTC	13	$NC_{(M)}$	15	12	42	36	80	60	95	105	26	56
AVG	200/3 667	1013/2 506		615/3 205	152/3 51	226/3 75	125/3 42	1102/3 367	1088/3 363	1095/3 365	189/3 63	78/3 26	191
PERS. HYGIENE													
Shower Floor	TNTC	TNTC	20	200	NC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	$NC_{(S)}$	691
Shower Wall	4	TNTC	NS	7	$NC_{(M)}$	TNTC	200	TNTC	90	19	TNTC	2	414
Head Floor	TNTC	TNTC	$NC_{(M)}$	54	112	150	TNTC	TNTC	170	110	$NC_{(M)}$	66	338
AVG	2004/3 668	3000 1000	20 20	2067/3 687	319/3 106	2150/3 717	2200/3 733	3000 1000	1260/3 420	1129/3 376	2110/3 703	68/2 34	529

Figure 3-21. Environmental Surfaces - According to Boat Area

ANDERSEN (AIR) SAMPLES (MICROORGANISMS/5 ft^3)

BASE LAB READINGS

MISSION DAY / SITE / PLATE-PARTICLE SIZE	-2 GALLEY SINK	2 FWD HEM	5 FWD HEM	8 FWD HEM	11	14 MIDBOAT	17	21	24	+8 FWD HATCH
1 > 9.2 μ	39	$NC_{(M)}$	TNTC	TNTC	20	40	$NC_{(S)}$	26	7	39
2 5.5 - 9.2 μ	26	11	6	46	$NC_{(M)}$	Broken Plate	$NC_{(M)}$	67	4	15
3 3.3 - 5.5 μ	31	NC	9	5	45	$NC_{(S)}$	$NC_{(M)}$	36	8	14
4 2.0 - 3.3 μ	1	110	4	33	72	Broken Plate	$NC_{(S)}$	85	2	19
5 1.0 - 2.0 μ	6	31	TNTC	TNTC	$NC_{(S)}$	47	$NC_{(M)}$	≈ 300	59	NG
6 < 1.0	1	152	NP	TNTC	12	120	NP	63	NP	NG
Total/5 ft^3	104							577	80	87
Avg Total/ft^3	20.8							115.4	16	17.4

ON BOARD READINGS

1		9	NG	17	9	8	NG	12		
2		1	2	NG	51	2	NG	NG		
3		> 100	1	NG	39	NG	NG	8		
4		1	NG	NG	4	8	6	164		
5		16	NG	120	NG	6	NG	> 100		
6		1	NP	4	NG	NG	11	30		
Total/5 ft^3		> 128	3	141	103	24	17	> 314		
Total/ft^3			< 1	28.2	20.6	4.8	3.4			

TYPES OF MICROORGANISMS RECOVERED

-2	2	5	8	11	14	17	21	24	+8
Micrococcus	A. niger	Pseudomonas	Bacillus	Pseudomonas	Achromobacter	Micrococcus	Aerobacter	Pseudomonas	Aerobacter
Sarcina	Mucor	Micrococcus	Aspergillis	Micrococcus	Micrococcus	Bacillus	Aspergillis	Archromobacter	Bacillus
Aspergillis	Micrococcus	B. anitratum	Nocardia	A. niger	Bacillus	Proteus	Micrococcus	Bacillus	Aspergillis
	Aerobacter	Aspergillis	Alternaria		Aspergillis	A. niger		Micrococcus	A. niger
		Alternaria				Alternaria		Aspergillis	Alternaria
		Yeast				Aspergillis		A. niger	
						Rhodotorula		Yeast	

LEGEND:

NP = No Plate Returned

NG = No Growth

TNTC = Too Numerous to Count

$NC_{(M)}$ = No Count - Moldy

$NC_{(S)}$ = No Count - Spreader

Figure 3-22. Andersen (Air) Samples

ENVIRONMENTAL SURFACES - GENERA ISOLATED

Location/Miss. Day	-7	-2	2	5	8	11	14	17	21	24	27	+6
Fwd. Hem. Floor	NS	Bacillus Micrococcus	Sarcina	Micrococcus	Aspergillis	Aspergillis	Bacillus S. aureus B. anitratum	Micrococcus	Bacillus Aspergillis	Micrococcus B. anitratum	Micrococcus Pseudomonas Aerobacter	Micrococcus Pseudomonas
Aft Hem. Floor	Bacillus Micrococcus	NS	Aspergillis	Aspergillis Micrococcus S. aureus	Sarcina	Micrococcus	Micrococcus	Micrococcus	Micrococcus Pseudomonas	Micrococcus Pseudomona	Micrococcus Bacillus Pseudomonas	Micrococcus Bacillus Pseudomonas
Galley Floor	Bacillus Micrococcus	Bacillus Micrococcus	Micrococcus	Micrococcus S. arueus	Micrococcus	Pseudomonas	Micrococcus Aerobacter	NGOT	Pseudomonas	Pseudomonas Micrococcus	Aerobacter	Aerobacter Pseudomonas
Shower Floor	Bacillus	Bacillus	Aspergillis S. aureus	Proteus	Micrococcus	Pseudomonas	Pseudomonas Proteus	Aerobacter Proteus B. anitratum	Aerobacter Pseudomonas	Aerobacter Proteus	Aerobacter Proteus	Aerobacter Micrococcus
Head Floor	Penicillium Bacillus Pseudomonas	Bacillus	A. niger	Aerobacter	NGOT	Micrococcus	Micrococcus B. anitratum	Proteus	Proteus	Micrococcus Bacillus	Aspergillis	Aspergillis Micrococcus
Fwd. Hem. Wall Port	Micrococcus Bacillus	Micrococcus Bacillus	NS	Micrococcus	Pseudomonas	Bacillus	Micrococcus	Pseudomonas	Bacillus	Bacillus	No Growth	No Growth
Fwd. Hem. Wall Stbd.	Micrococcus	Micrococcus Bacillus	A. niger	Aspergillis	No Growth	No Growth	Pseudomonas	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Aft Hem. Wall	Bacillus	Bacillus Micrococcus Aspergillis	Alternaria	Aspergillis	Aerobacter	B. anitratum	Aspergillis	Micrococcus	Micrococcus Aspergillis B. anitratum	No Growth	No Growth	No Growth
Shower Wall	Micrococcus	Bacillus	NS	Micrococcus Yeast (Budding)	Aspergillis	Aerobacter	Aerobacter	Pseudomonas	Aerobacter Pseudomonas	Micrococcus Aerobacter	Pseudomonas	Pseudomonas Aspergillis
Galley Sink Formica	Micrococcus Bacillus Nocardia	Micrococcus Bacillus Sarcina	Micrococcus	Aspergillis	NGOT	Micrococcus	Micrococcus Bacillus	Micrococcus	NGOT	Pseudomonas	Pseudomonas Micrococcus	Aspergillis Mucor
Galley Sink Stainless	Micrococcus Bacillus	Micrococcus	Aspergillis	Aspergillis	Pseudomonas	Micrococcus	Micrococcus Bacillus S. aureus	Bacillus	No Growth	Micrococcus Pseudomonas	Bacillus	Micrococcus
Ward Rm. Table Top	Penicillium Micrococcus	Sarcina Micrococcus	A. niger	Aspergillis Micrococcus	Aspergillis	Micrococcus	Micrococcus S. aureus B. anitratum	Micrococcus Pseudomonas B. anitratum	Micrococcus Bacillus B. anitratum	Micrococcus	Micrococcus Aspergillis	Micrococcus Aspergillis Bacillus
Bench Top Port	Bacillus Micrococcus	Bacillus Micrococcus	Mold	Aspergillis Micrococcus	Micrococcus	Micrococcus	Micrococcus Aerobacter B. anitratum	Micrococcus Bacillus Pseudomonas	Aspergillis Micrococcus Pseudomonas	Aspergillis Micrococcus B. anitratum	Micrococcus Pseudomonas	Aspergillis Micrococcus
Head Seat	Micrococcus	NS	Micrococcus S. aureus	NS	NS	NS	NS	NS	NS	NS	NS	Micrococcus S. aureus
Garbage Bin	Penicillium Aspergillis Bacillus	Bacillus	S. aureus	Alternaria	Bacillus Aspergillis	Micrococcus	Bacillus B. anitratum	Bacillus Micrococcus Aspergillis	Micrococcus	Bacillus Pseudomonas	Bacillus Micrococcus B. anitratum	Aspergillis

Figure 3-23. Environmental Surfaces - Genera Isolated

ENVIRONMENTAL SURFACES ADDITIONAL POST-MISSION SAMPLES

Area	No. microorg/4 Sq. In.	Types Found
Garbage Bin #2 Top - Bag	1	NGOT
Garbage Bin #1 Top - Bag	2	Mucor B. anitratum
Fwd. Hem. Wall - Front	2	Aspergillis Micrococcus
Aft Escape Hatch - Inside	5	Mold Bacillus
Ward Rm. Seat Back	15	Aspergillis, Yeast Micrococcus
Galley Formica	TNTC	Bacillus Micrococcus
Fwd. Hem. Floor Under Ladder	TNTC	Bacillus Micrococcus
Pilots Console	$NC_{(M)}$	Pseudomonas Mucor Micrococcus
Fwd. Hem. Floor Under Seat	47	Micrococcus Pseudomonas
Bracket - Rear Hemis.	31	Pseudomonas Micrococcus

Figure 3-23. Environmental Surfaces - Genera Isolated (Sheet 2 of 2)

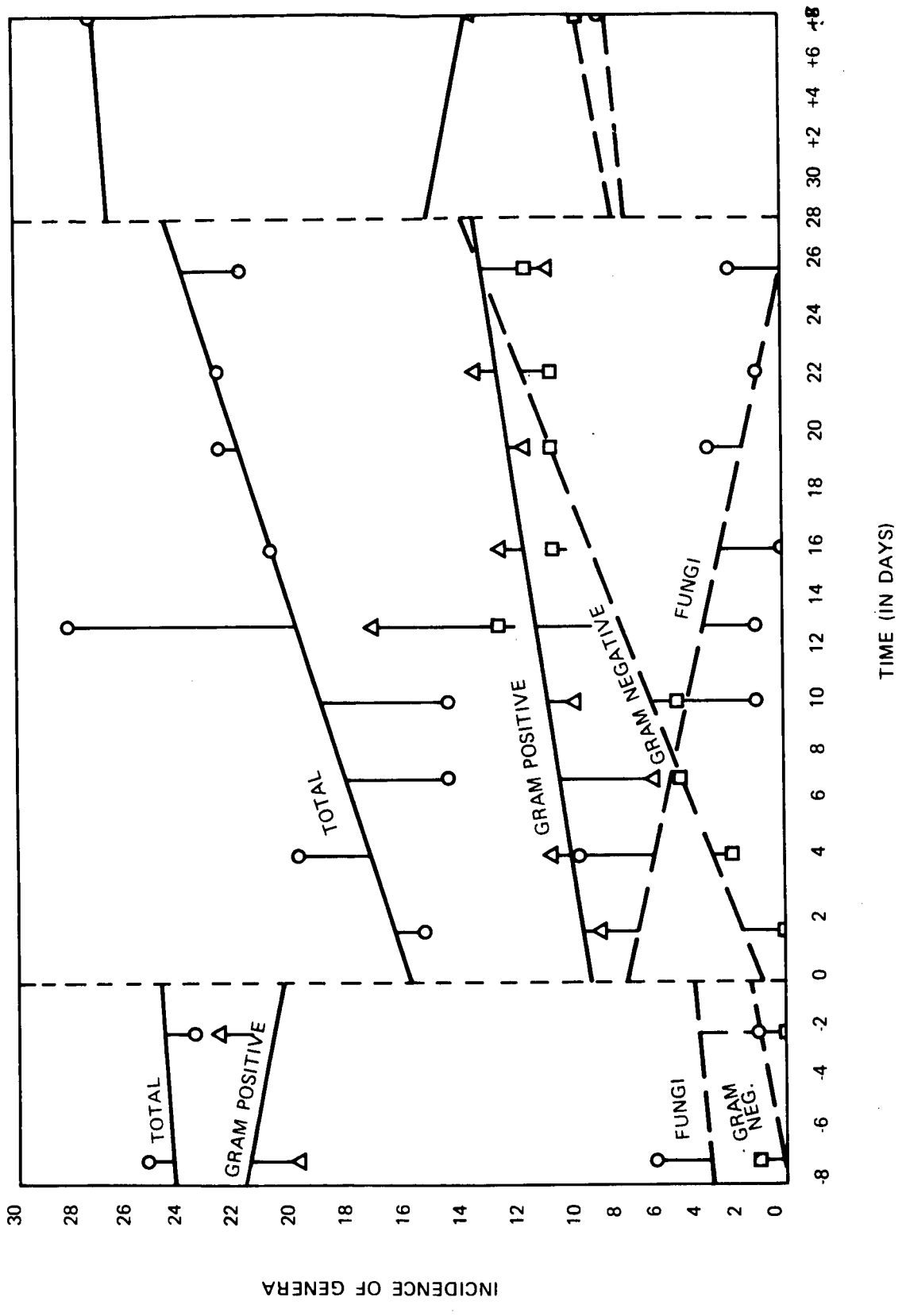

Figure 3-24. Shift of Surface Flora

BEN FRANKLIN ENVIRONMENTAL FLORA

	Pre-Mission	Mission	Post-Mission	Total
Total Samples Taken	27	125	25	177
For Microbiology	27	125	25	177
Chemical Analysis	0	0	0	0
Total Number of Isolates	49	175	45	269
Total Colonies Picked	71	250	95	416
Total Number of Sterile Samples	0	6	2	8
On Board Readings	N/A	50	N/A	
Laboratory Readings	0	6	2	8
Total Samples Lost	0	4	0	4
In Shipment	0	0	0	0
In Storage (Too Long)	0	4	0	4
Total Number of Isolates Identified to Genus	49	174	44	266
Total Number of Genera Found	7	12	9	15
		Plus 1 Unidentified Mold	Plus 1 Unidentified Mold	Plus 2 Unidentified Molds

Figure 3-25. Summary: Ben Franklin Environmental Flora

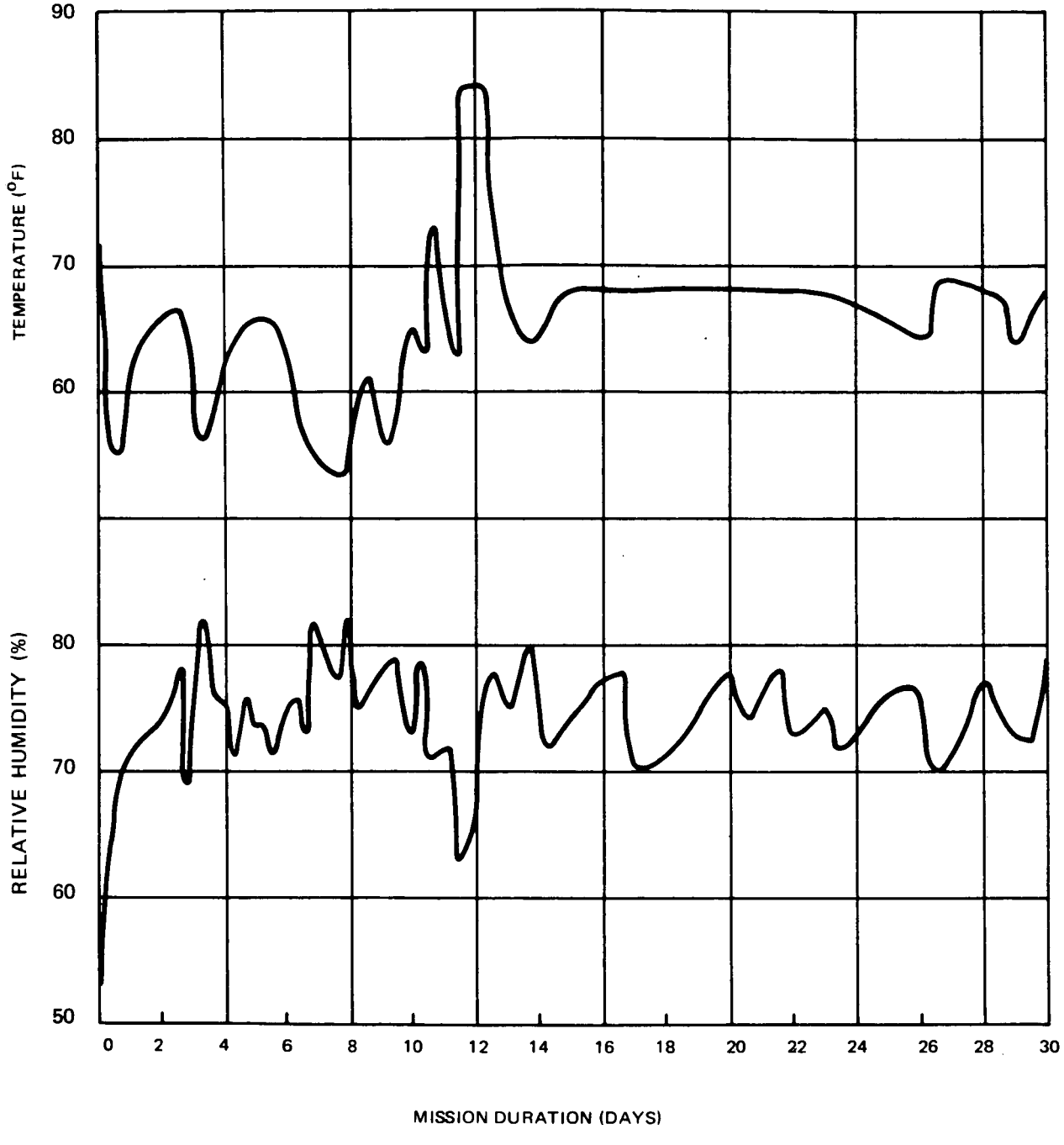

Figure 3-26. Temperature and Relative Humidity: Mission Profile

both Aerobacter and Proteus. B. anitratum, whose significance is discussed in Subsection 3.1 and which apparently was from human sources, appeared in the air on Day 5 and was subsequently recovered frequently from the surfaces. Staph aureus was also recovered from surfaces at the start of the mission, but not in pre-mission samples. Since it was not present in pre-mission cultures, it can be assumed the source was the human carriers. It is interesting that Staph aureus was never recovered from the air samples, even though it was on the men and the environmental surfaces, since transmission through the air is common.

The increasing presence of human associated organisms which were not present at pre-mission sampling, leads one to feel that initially, in the interactions between the men and the environment, the men exerted the stronger influence. It is entirely possible that any spread of microorganisms from man-to-man was mediated through the common environment. However, proof would require an in-depth epidemiological study with detailed strain typing of bacteria. Clean room experiences (Ref. 16), where the environment is strictly controlled, indicate that the types of organisms recovered are mainly of indigenous human origin.

The shift towards gram negative rods can be noticed by inspection of Figure 3-23 and is graphed in Figure 3-24. While this shift truly occurred based on the bacteria recovered, it must be viewed in the light of the low incubation temperatures, the long storage time of samples, and the use of antimicrobials, all of which favored the recovery of gram negative organisms. Evidence for the selective effect of the antimicrobials is reenforced by inspection of Figure 3-17 where it can be noted that after the washing on Day 14 the S. aureus (gram positive) disappeared from the environment but the Pseudomonas (gram negative) spread rapidly.

Figure 3-24 indicates a decrease in the fungi on surfaces which agrees with results from submarine studies (Ref. 17). However, in the atmosphere samples no decrease in fungi was apparent.

Several miscellaneous items were tested, also.

- The garbage bin, which was sprayed daily with the quaternary amine solution, maintained a bacterial population of the same order of magnitude as the table surfaces which were washed daily. This method of treatment was apparently sufficient to maintain a low level of contamination in the garbage bin
- Various absorbants which were returned to the base laboratory at the end of the mission were tested for a qualitative estimate of the microorganisms contained in or on them. From the charcoal bags and absorbant panels only Bacillus was recovered. From the Protec-sorb bags, Bacillus and Aspergillis were obtained.

A general summary is shown in Figure 3-25. **Figure 3-26 presents a temperature** and humidity profile.

3.2.3 Conclusions

- As the mission progressed, environmental flora and that of the men became similar. Both showed an apparent shift towards gram negative organisms
- Cleaning resulted in a transient decrease of contamination levels followed by a rapid resurgence, particularly evident on the floors
- No direct relationship could be determined between air and surface contamination levels.

3.2.4 Recommendations

- Re-evaluate cleansing protocols - possibly wash all areas daily or do not wash at all
- Devise different approach to body washing - eliminating conventional shower.

3.3 WATER SYSTEM

The water system has a history of test data dating back to December 1968 which indicated a persistent bacterial contamination problem. Continued contamination problems led to seven unsuccessful attempts at system sterilization and ultimately the adoption of the final pre-mission protocol:

- Detergent wash of 0.1% Triton X-100 and 0.1% sodium pyrophosphate with a total volume of 350 gallons. This was filtered through the Filtration Rig (FR) which consisted of a 3-micron roughing filter and a 0.1 micron final filter, prior to filling the cold water system
- Following an overnight soak, the detergent solution was pumped overboard and the system rinsed by refilling it twice with filtered water
- A 75-ppm Iodine solution (350 gals.) was then put on board through the filter rig. This was allowed to remain overnight and then pumped overboard
- A sterile (filtered) nitrogen purge was used to remove iodine vapors
- The cold water system was then filled with fresh, low mineral content, water to which iodine was added to give a concentration of 7.5 ppm
- Hot water tanks were loaded with filtered, non-iodinized water.

3.3.1 Results

Figures 3-27 and 3-28 provide a complete tabulation of the pre-mission, mission and post-mission results. It is evident that the quality of the cold water from the time of initial loading did not meet the criterion of sterility as required for space water systems. However, the low level of contamination and the absence of coliform bacteria made it potable by ordinary standards. It should be noted that by Day 5 of the mission, when the first positive water monitor was observed, use of the cold water for drinking and food reconstitution was stopped and only the hot water source was used for these purposes. Early in the mission (Day 8) coliforms appeared in the cold water system (making it non-potable by any standard) along with other human associated organisms. On Day 23 E. coli was recovered in the head sink sample.

By the time post-mission samples were obtained, bacterial levels were at about 10^4 ml in the cold water system, and the character of the contaminants had reverted to mainly Pseudomonas which had been the persistent pre-mission problem. Since no numerical data are available for mission samples, the increase in numbers of bacteria cannot be related to specific events or plotted as a function of time.

WATER SYSTEM MONITORING RESULTS

PRE-MISSION

DATE	LOCATION - TANK	STERILITY TEST (72 Hr. Reading) FTG	SLM	TGE	ORGANISMS/ML (TGE)	ORGANISMS RECOVERED	IODINE (ppm)	TDS (ppm)	COD ppm	CHLORIDE ppm	AMMONIA	PHOSPHATE ppm
7-12	Tanker (as rec'd)	+	+	+	300	Sample Lost						
(-2)	Galley Sink - 1						0.4 I_2 ppm					
	Head Sink - 1						0.2					
	Shower Sink - 1						0.5					
	Shower DISP - 1						0.6					
	HS - 2		STRONG I_2 STERILIZING SOLN-75ppm I_2				0.7					
	HS - 3						2.7					
	HS - 4						9.6					
7-13	Tank - 1						45					
(-1)	Tank - 2						50					
	Tank - 3		PUMP OFF OF STRONG I_2 SOLN				44					
	Cold Water Loaded On Board With 7.5 ppm I_2 Hot Water Loaded On Board With No Iodine											
7-14	GS - 1	+	+	+	400	Pseudomonas	0.5	56	1212	32	0.01	0.1
(0)	HS-1	+	-	+	150	Pseudomonas	0.3		2697	5.6	0.01	0.1
	SS-1	+	+	+	150	Achromobacter	0.75		1029	30.0	0.08	0.3
	SD-1	+	+	+	150	Pseudo, Flavobacterium	0.4		1626	2.0	0.08	0.3
	GS-2	+	•	+	75	Pseudo	0.2	51	1686	1.8	0.01	0.1
	GS-3	+	+	+	25	Pseudo	0.2	51	1649	1.0	0.01	0.1
	GS-4	•	-	+	3	Pseudo	0.7	51	1561	1.0	0.04	0.15
	HOT WATER											
	Tank-1 90°	-	-	-		None (Sterile)		7	6.8	0.1	0.01	0.15
	Tank-2 90°	-	-	-		None (Sterile)		7	6.8	0.1	0.01	0.15
	Tank-3 90°	-	-	-		None (Sterile)		7	2.0	0.1	0.01	0.1
	Tank-4 90°	-	-	-		None (Sterile)		7	2.0	0.1	0.01	0.1

Figure 3-27. Water System Monitoring Results (Sheet 1 of 2)

WATER SYSTEM MONITORING RESULTS

POST-MISSION

DATE	LOCATION - TANK	STERILITY TEST (72 Hr. Reading) FTG	SLM	TGE	ORGANISMS/ml (TGE)	ORGANISMS RECOVERED	REMARKS	TDS (ppm)	COD ppm	CHLORIDE ppm	AMMONIA	PHOSPHATE ppm
8-22	GS-1 Line			+	1.6×10^4	Achromobacter			1725	0.8	0.05	0.1
(+8)	HS-1 Line			+	7.7×10^4	Pseudomonas			1382	1.2	0.04	0.15
	SS-1 Line			+	1.8×10^4	Pseudo			1635	2.0	0.04	0.1
	SD-1 Line			+	6.6×10^4	Pseudo			1790	1.4	0.01	0.1
	GS-1			+	5.9×10^4	Micrococcus, Pseudo			1725	1.8	0.01	0.1
	HS-1			+	3.0×10^4	Pseudo			1820	1.8	0.01	0.1
	SS-1			+	2.4×10^4	Micrococcus, Pseudo			1782	1.6	0.01	0.1
	SD-1			+	1.1×10^4	Pseudo			1637	1.8	0.01	0.1
	GS-2			+	1.2×10^4	Pseudo			1784	9.0	0.03	0.1
	GS-3			+	2.5×10^1	Pseudo			1778	5.0	0.05	0.1
	GS-2 HOT			+	1.4×10^4	Micrococcus, Pseudo	Ambient Temp		2.0	0.3	QNS	0.15
	GS-3 HOT			+	3.3×10^2	Pseudo	Ambient Temp		9.5	0.2	0.01	0.15
	GS-4 HOT			+	3.5×10^2	Pseudo	Ambient Temp		2.0	0.1	0.01	0.1
	TANK-1 HOT	NO READING - TANK DRY										
8-26 (+12)	GS Filter HS Filter SS Filter	RODAC PLATES			GROSS CONTAM.	Pseudomonas						

* FTG = Fluid Thioglycollate

SLM = Sabarouds Liquid Medium

TGE = Tryptose glucose extract Agar

Figure 3-27. Water System Monitoring Results (Sheet 2 of 2)

WATER SYSTEM MONITORING RESULTS

MISSION DATA

DATE	DAY	LOCATION - TANK	72 HOUR READING MILLIPORE KIT (1) ENDO	TOTAL	YEAST-MOLD	ORGANISMS RECOVERED AT BASE LAB FROM MILLIPORES (2)	REMARKS
7-14	0						I_2 reading = 0 No further readings made. No reapplications of Iodine.
7-15	1	GS-1	-	-	-	Pseudomonas, Mycelia sterilia	
7-16	2	GS-2	-	-	-	NGOT	
		SS-2	-	-	-	Pseudo, Cladosporium	
		HS-3	-	-	-	Pseudo Clado, Achromobacter	
7-17	3	GS-2	-	-	-	Clado	
7-18	4	GS-2	-	-	-	Pseudo, Clado Achromo	
7-19	5	GS-2	-	-	-		
		HS-2	-	-	-	Bacillus, Mycelia sterilia	
		SS-2	-	-	-	NGOT	
7-20	6	GS-2	-	-	-	NGOT	
7-21	7	GS-2	-	-	-	Pseudo, Clado	Changed on-line filters at all sinks.
7-22	8	GS-2	-	-	-	Micrococcus, Clado, Mucelia sterilia	
		HS-2	-	-	-	Aerob.	
		SS-2	+	+	-	Pseudo, Clado, Aerob.	

Figure 3-28. Water System Monitoring Results (Sheet 1 of 3)

WATER SYSTEM MONITORING RESULTS

MISSION DATA (Cont.)

DATE	DAY	LOCATION - TANK	72 HOUR READING MILLIPORE KIT (1) ENDO	TOTAL	YEAST-MOLD	ORGANISMS RECOVERED AT BASE LAB FROM MILLIPORES (2)	REMARKS
7-23	9	GS-2	-	-	-	Not recd.	
7-24	10	GS-2	-	-	-	NGOT	
7-25	11	GS-2	-	-	-	Clado, Aerob	
		SS-2	+	-	-	Pseudo, Clado	
		HS-2	+	.	+	Aerob	
7-27	13	GS-4 HOT	-	-	-	Clado, Bacillus	
		GS-3	-	-	-	Clado, Aerob, Mycelia sterilia	
7-28	14	GS-3	-	-	-	NGOT	
		SS-3	-	+	-	B. anitratum, Clado	
		HS-3	.	.	+	Pseudo, Aerob	
7-29	15	GS-3	-	-	-	Aerob, Penicillum	Changed on-line filters at all sinks.
7-30	16	GS-3	.	.	.	Pseudo, Aerob, Clado	
7-31	17	GS-3	-	-	-	NGOT	
		SS-3	-	-	-	Aerob, Clado	
		HS-3	.	.	+	Pseudo, Citrobactor	
8-1	18	GS	-	-	-	NGOT	
8-2	19	GS	-	-	-	NGOT	
8-3	20	GS	.	.	-	Pseudo, Clado	
		HS	+	+	-	Proteus	
		SS	.	-	-	Aerob	

Figure 3-28. Water System Monitoring Results (Sheet 2 of 3)

WATER SYSTEM MONITORING RESULTS

MISSION DATA (Cont.)

DATE	DAY	LOCATION - TANK	72 HOUR READING MILLIPORE KIT (1) ENDO	TOTAL	YEAST-MOLD	ORGANISMS RECOVERED AT BASE LAB FROM MILLIPORES (2)
8-4	21	GS	+	-	-	NGOT
8-5	22	GS	+	+	-	NGOT
8-6	23	GS	+	+	-	NGOT
		HS	+	+	-	E. coli, Pseudo
		SS	+	+	-	Pseudo, Clado
8-7	24	GS	-	-	-	Achromo
8-8	25	GS	+	-	-	NGOT
8-9	26	GS	+	-	-	Aerob, Clado
		HS	+	+	-	Pseudo, Citrob
		SS	+	+	-	Pseudo
8-10	27	GS	NO RECORD			Pseudo
7-30	16	GS				Aerobacter (4.7×10^6)[3]
		HS				Pseudo Aero (5.8×10^7)[3]
		SS (2 samples)				Pseudo (1.3×10^5)[3]

(1) Monitors incubated at ambient boat temp (≈67° F).

(2) Plated out at base lab after storage on-board at ambient temp. for duration of mission.

(3) In bottles received several weeks post-mission.

GS = Galley Sink

HS = Head Sink

SS = Shower Sink

SD = Shower Dispensor (shower head)

NGOT = No growth on transfer

Figure 3-28. Water System Monitoring Results (Sheet 3 of 3)

Results on the hot water tank are rather sparse since this system was not tested during the mission. Pre-mission sampling, when the tanks were hot, indicated no viable bacteria. However, in post-mission samples, when the tanks were at ambient temperature, contamination levels resembled those of the cold water system.

3.3.2 Discussion

The presence of bacteria from Day 0 of the mission showed the inadequency of the sterilizing protocol. The choice of a 75-ppm I_2 sterilizing solution was based on LM experience and entailed many compromises between killing ability, interactions with construction materials and potability. Studies reported in Applied Microbiology (Ref. 8) indicate a betadine solution (providone-iodine) of 0.05% (500 ppm) was required to kill a 10^4 organisms/ml solution of Pseudomonas aeroginosa in sterile tap water. The original concentration of Pseudomonas in the tanks, lines and taps was not known, but the 75 ppm, effective on LM, was obviously not sufficient in this case, possibly due to a higher initial bacterial load.

It must be noted that in contrast to spacecraft systems, the GSDM system was constructed with dead-ends, places of entrapment and iodine absorbing plastic materials which are incompatible with a system where residual iodine is supposed to be used for sterility maintenance. Also, these results reflect not the condition in the tanks themselves, but rather the quality of water emerging from the taps after passage through the lines. The rapid iodine depletion is apparent from the data presented in Figure 3-27. Not only was the I_2 level considerably lower in the pump off of the 75 ppm solution, but the levels as recorded at the taps are much below the desired residual of 7.5 ppm.

Although provision was made for reiodization of the cold water system, no such attempts were made when the initial mission reading indicated zero level. Because of this, iodine treatment of the water cannot be truly evaluated since a persistent iodine level may have maintained the system, at least at a low contamination level.

In addition, Favero and Drake (Ref. 19) in comparing chlorination and iodization as sanitary control in swimming pools discovered that in pools treated with iodine, Pseudomonas accounted for most of the microbial flora and showed that the accumulation

of large numbers of these bacteria was due to their iodine resistance and ability to grow rapidly in the absence of free iodine. Possibly, the initial loading of Pseudomonas in the water system made iodine sterilization a less desirable choice.

It is apparent also, that the bacterial filters fitted on the delivery lines and air vents as back-up to the iodine treatment became contaminated themselves and did little if anything to prevent the spread of bacteria. In fact, the first instance of coliform contamination occurred just after the first change of filters. The changes in the nature of bacterial contamination in the water is interesting in that during the mission it increasingly reflected human associated organisms. One can speculate that this is due to contamination of the taps through handling which was then carried along with the water being sampled. It may be necessary to design into the system provision for eliminating this outside-to-inside type of contamination.

Discrepancies between onboard reading of millipore monitors and subsequent evaluation of these same monitors upon their return to the base laboratory presents a serious problem of water quality assurance. There are even discrepancies among the onboard readings, since any positive result on the Endo medium (for coliforms) should be accompanied by a positive result on the Total medium. A scan of the onboard readings (Figure 3-28) indicates that this is not always the case. There are several possible reasons for these divergences:

- Inhibition of growth by undetected residual iodine in the water
- Growth manifesting itself as a thin film might not have been visible in the closed monitor to an untrained observer, particularly under the poor lighting conditions appearing in the GSDM
- Low incubation temperatures (64 to 68^{o}F) resulted in slow growth rate and, therefore, not all colonies were visible at 72 hours
- Regarding the onboard discrepancies, there were manipulative difficulties associated with the application of growth medium to the monitors. One can speculate that some of the contradictory readings (such as positive on Endo and negative on Total) may have resulted from such problems.

It must be emphasized that the millipore monitors were designed for use with incubation temperatures of around 95°F, not the ambient boat temperatures at which they were incubated.

Although coliforms are the standard marker organisms for failure of water potability, Pseudomonas contamination proved to be the most intractable problem in the GSDM system because of its widespread nature and resistance to all attempts at eradication.

According to Farmer and Herman (Ref. 20), "In this decade, Pseudomonas aeroginosa is replacing Staph aureus as the greatest concern in hospital-incurred infections. Suppression of the normal flora by antimicrobial agents and depression of the normal host defense mechanisms by immunosuppressive and other agents have made many members of today's hospital population prime targets for Pseudomonas aeroginosa invasion. Pseudomonas aeroginosa has an exceptional ability to survive and multiply in the hospital environment and is often cultured from hand creams, mop buckets, sinks, sterile solutions, water baths, humidifiers, and similar ecological niches." Pneumonias and very serious bacteremias have been caused by Pseudomonas and sources of infection have included water environments, other individuals and Pseudomonas infections, and fecal material from normal individuals.

Much of the above is applicable to a closed environment, particularly for the GSDM where antimicrobials were used and the normal body flora appeared to be altered. Extrapolation to long term mission situations where a lowered immune status is postulated, makes the presence of Pseudomonas in the water an extremely serious problem inasmuch as the water system provides such an excellent means of dispersion. In addition, Pseudomonas can produce off-odors and tastes which would render the water esthetically undesirable.

A discussion of the water chemistry is presented separately in Appendix B. Unfortunately, chemical data is available only for pre- and post-mission samples. It can be noted, however, that great differences in chemical oxygen demand (COD) can be related to the hot versus cold water rather than the bacterial contamination levels indicating the tank construction material was responsible for much of the organic loading, (high COD's being noted in the cold water samples which came from epoxy painted tanks). The hot water, which was stored in metal tanks gave the low COD levels.

A summary of water sampling is presented in Figure 3-29.

3.3.3 Conclusions

- Discrepancies between onboard and base laboratory data indicate a potentially serious hazard
- The inability to detect contaminated cold water during the GSDM points out the need for improved methods and techniques to identify microbes onboard.
- Iodine was not given a fair trial. The original sterilizing solution may have been too low and there was no monitoring or reapplication by the crew.

3.3.4 Recommendations

- Redesign of the monitoring system is required. An automated onboard system with capacity to count viable bacteria and identify to genus, is a possibility
- Re-evaluate the millipore monitors
- Investigate the ability of hot water to remain potable during long periods of storage
- Re-evaluate iodine with strict adherence to protocol
- Redesign the filters to include:
  - medium with lower mu rating
  - different boss and gasket
  - packaging under sterile conditions
  - in-situ sterilization capability
  - augmentation with silver ion generators
- Redesign the water management system to provide:
  - biocompatible materials
  - all metal, SST
  - recirculation capability
  - avoid stagnation areas

BEN FRANKLIN WATER SAMPLES

	Pre-Mission (1)	Pre-Mission (2)	Mission	Post-Mission	Total
Total Samples Taken	23	244	139 (3)	13	396
Total Samples Taken For:					
Microbiology	12	189	139	13	341
Chemical Analysis	18	55	4	13	72
Total Number of Isolates	8	74	64	16	154
Total Number of Sterile Samples	4	64	9	0	73
Onboard Reading	N/A	N/A	24 (on all media)	N/A	N/A
Laboratory Reading	4	64	9	0	73
Total Samples Lost	1	45	4	0	49
In Shipment	1	27	0	0	27
Too Long Storage	0	18	4	0	22
Number of Isolates Identified to Genus	8	74	64	16	154
Number of Genera Found	3	7	12	3	16

(1) Tests on water loaded for GSDM.

(2) All testing done to qualify water system (12/6/68 - 7/5/69).

(3) 45 samples x 3 millipores each + 4 bottles recovered postmission.

Figure 3-29. Summary: Ben Franklin Water Samples

3.4 WASTE

The waste management system consisted basically of a marine toilet, macerator, and holding tanks. Water for flushing came from the mini-waste tanks which held waste water from washing. Provision was made for the automatic addition of 1 ounce of iodine-phosphoric acid germicide (Wellodyne) per flush operation. In addition, varying amounts of a quaternary amine antimicrobial (Microgard) were added throughout the mission (Figure 2-1).

The history of this system dates back to December 1966 when a prototype unit was evaluated in a 5-day, 2-man, chamber test with the iodine germicide used alone. Growth and odor were inhibited in the holding tanks where the pH was 2.45 but not in the lines where the pH rose to 6.35 and no residual iodine was detected.

Additional data were obtained during pre-mission sea trials. The system was used intermittently for 13 days with the iodine germicide, at which time odors were noticed coming from the mini and main waste tanks. At that time 4 ounces of the quaternary amine was added to each tank. This appeared to stop the odor problem. Nine days later, after another series of test dives and intermittent use of the system, the waste was pumped off and microbiological samples taken. Results using a plate count method with Letheen Agar showed that the main waste tanks contained no viable bacteria.

During the mission, problems were encountered at various times with the iodine dispenser, blower, and macerator culminating in the complete breakdown of the macerator on Day 29.

No samples were taken during the mission, but the waste tanks were sampled upon the boat's return to shore. Results of this sampling are tabulated in Figure 3-30. A summary of the sampling is presented in Figure 3-31.

The absence of mission data points seriously limits the evaluation of the effectiveness of waste treatment procedures. The observation of odors during the mission, with decision to increase the antimicrobials gives some indication that the disinfecting agents were not working satisfactorily.

POST-MISSION WASTE WATER

DATE	SOURCE	ORGS/ML	TYPE RECOVERED	CONDUCT (TDS) ppm	COD ppm	(NH_4)	(PO_4)		TREATMENT CONDITIONS DURING MISSION
8/22	Mini Waste Tank Bottom	1.1×10^6	Bacillus Micrococcus	9250 (5920)	20693	1040	740	1260	1 8 oz. pre-charge of quat. 2 Washwater contained Antimicrobial Soap
	Middle	9.6×10^6	Micrococcus Gram Neg Rod	9500 (5803)	19307	1120	500	1260	3 Various Areas, Plates and Sinks Cleaned With Quat. Amine
	Top	2×10^7	Micrococcus E. coli	9250 (5920)	19230	1160	500	1260	
	Main Waste #1 Bottom	9×10^6	Proteus Micrococcus	10300 (6592)	19597	1320	560	1080	1 Pre-Charge 4 ox. Quat/Tank 2 50cc Wellodyne per flush
	Middle	1.3×10^6	Micrococcus	10400 (6656)	19421	1280	560	1080	3 2 oz ev. 3rd day in head
	Main Waste #2 Bottom	6.5×10^6	Micrococcus E. coli	10,000 (6400)	19307	1296	720	860	4 Additional Quat. when odor noticed (see activity chart)
	Main Waste #3 Bottom	1.2×10^7	Micrococcus Bacillus	10400 (6656)	19497	1280	550	1140	
	Main Waste #4 Bottom	1.1×10^7	Micrococcus Gram Neg Rod	9900 (6336)	25590	1408	350	1020	
	Galley Sink Trap	4.5×10^8	Pseudomonas	QNS*	4731	124	75	7600	
	Head Sink Trap	6×10^5	Pseudomonas	QNS	2951	56	64	35	
	Shower Sink Trap	$>10^6$	Aerobacter	QNS	QNS	QNS	QNS	QNS	
	Toilet Water	4×10^4	Pseudomonas	8750 (5600)	11805	760	550	1300	
8/26	Galley Filter Bowl	4.1×10^3	Pseudomonas	QNS	1417	QNS	QNS	QNS	
	Head Filter Bowl	$>10^6$	Pseudomonas	QNS	1249	QNS	QNS	QNS	
	Shower Filter Bowl	$>10^6$	Proteus	110 (70.4)	1647	0.08	0.2	3.75	
	Bilge Water	$>10^6$	Micrococcus Proteus	7000 (4480)	14531	1180	475	1640	

*QNS = Quantity Not Sufficient

Figure 3-30. Post-Mission Waste Water

WASTE SAMPLES

	Pre-Mission	Mission	Post-Mission
Total Samples Taken For:	N/A	N/A	13
Microbiology			13
Chemical Analysis			13
Total Number of Isolates			24
Total Number of Sterile Samples			0
On Board Readings			N/A
Laboratory Readings			0
Total Samples Lost			0
in Shipment			N/A
in Storage (too long)			N/A
Total Number of Isolates Identified to Genus			22
Total Number of Genera Found			6(1)

(1) Plus 2 unidentified gram negative rods

Figure 3-31. Summary: Waste Samples

Upon post-mission sampling all waste tanks were found to be grossly contaminated with 10^6 to 10^7 microbes/ml. At this point the system had obviously failed microbiologically. The complete failure of the macerator, which prevented proper mixing of germicide and waste, added to the problem of decontaminating the waste.

The organisms recovered from the waste tanks were the usual human enteric bacteria; Proteus, E. coli, gram negative rods, micrococcus, and the very common Bacillus. It is interesting that among those organisms recovered were the strong odor and gas producers, Proteus and E. coli, thus possibly explaining the origins of odors.

In contrast, waste water from the sink traps and filter bowls reflected bacteria found in the cold water system, mainly Pseudomonas.

In a holding tank type waste management system, microbiological attention is focused on two problems - inhibition of bacterial growth and control of odors. The second problem is partially solved by controlling the bacterial growth. In the BEN FRANKLIN, neither problem was ultimately solved. There can be several possible explanations for the microbiological failure of the system:

- Mechanical breadown of the macerator prevented proper mixing of waste and inactivating agents
- Excessive depletion of residual iodine occurring in tanks
- Improper or inadequate addition of germicides
- Incompatibility of germicide mixtures i. e. iodine and phosphoric acid with quaternary amine and tin complex.

The literature (Ref. 21) indicates that one of the chemicals incompatible with quaternary amines is iodine. Not only was the wash water containing quats used for flushing the toilet, but the quat mixture was deliberately added to the waste tanks. This may explain why the system worked during the chamber run when only the Wellodyne was used, but problems developed with the combination of agents.

3.4.1 Conclusions

- The waste management system as designed was inadequate microbiologically
- Organisms from waste tanks were generally human (mainly enteric) in origin as contrasted with those from filter bowls and traps.

3.4.2 Recommendation

Re-evaluate the waste system mechanically and for choice and concentration of inactivating chemicals.

3.5 FOOD MICROBIOLOGY

Foods selected for the GSDM were either commercially canned or freeze dried. The latter was supplied in plastic bags repackaged from bulk.

Only the repackaged foods were tested microbiologically, based on the assumption that foods from regular commercial suppliers had met Federal or State standards.

Results of sampling are presented in Figure 3-32. At the time of pre-mission sampling, the food supply was approximately 12 weeks old. Post-mission sampling was performed approximately 12 weeks later.

In a majority of the 30 food samples, pre-mission microbial contamination was below detectable limits, and only 2 items had counts greater than 2000/gm. All but two of those with detectable microbial growth contained the single genus Bacillus, a common saprophytic contaminant.

Post-mission sampling of available food packages revealed only small differences in microbial load which may be attributable to package-to-package variation. The only outstanding sample was the tomato juice (15,000 organisms/gm). Unfortunately no pre-mission sample was available for comparative purposes.

The absence of coliforms and the generally low microbial contamination levels indicate food of adequate quality.

While a universal, mandatory standard for bacteriologic food acceptability is not available, recommended standards of 50,000 to 100,000 organisms/gm have been

FOOD MICROBIOLOGY

SAMPLE #	ITEM (1)	PREMISSION		POSTMISSION (2)	
		NUMBER ORGANISMS/gm	TYPE	NUMBER ORGANISMS/gm	TYPE
1	Orange Drink	<5			
2	Pineapple Crystals	<5			Yeast
3	Choc. Milk Shake	2500	Aspergillus		
4	Milk Non-Fat Instant	500	Bacillus		
5	Beef Soup, Instant	2400	Bacillus		
6	Chicken Soup	700	Bacillus		
7	Peas, Freeze Dried	85	Bacillus		
8	Familia With Milk & Sugar	200	Bacillus	250 (Opened Pkg.)	Bacillus
9	Peaches	<5			
10	Butterscotch Pudding	90	Bacillus	2400	Bacillus
11	Grape Punch	<5		<5 (Opened)	
12	Grapefruit Crystals	<5		<5	
13	Pink Lemonade	<5			
14	Chicken Salad	50	Unidentified		
15	Scrambled Eggs, Instant	<5			
16	Egg Salad	90	Bacillus		
17	Pea Soup, Instant	480	Gram Neg Rod (Not Coliform)		
18	Tuna Salad	150	Bacillus		
19	Orange Crystals	<5			
20	Apple Sauce, Instant	<5		<5	
21	Mashed Potatoes, Instant	295	Bacillus		

Figure 3-32. Food Microbiology (Sheet 1 of 2)

FOOD MICROBIOLOGY (Cont.)

SAMPLE #	ITEM (1)	PREMISSION		POSTMISSION (2)	
		NUMBER ORGANISMS/gm	TYPE	NUMBER ORGANISMS/gm	TYPE
22	Fig Newtons	200	Bacillus		
23	Chocolate Pudding	170	Bacillus	710 (Closed)	Bacillus, Mold
				1020 (Opened)	Bacillus, Mold
24	Beef & Rice	<5			
25	Carrots, Diced	<5			
26	Beef Stew	<5			
27	Cold Cereal	<5			
28	Cookies	<5			
29	Fruit Cocktail	<5		30	Bacillus
30	Peas & Carrots	<5			
31	Tomato Juice	N.S.		15,000	Bacillus
32	Bacon Bar	N.S.		50 (Opened)	Bacillus

(1) A different package was sampled for each determination (Pre and Post mission)

(2) May reflect: Differences during packaging
Effects of storage for 30 days

LEGEND

< = Less Than

> = Greater Than

N.S. - No Sample

Figure 3-32. Food Microbiology (Sheet 2 of 2)

SUMMARY

#ORGS/GM	2000	1500-2000	1000-1500	500-1000	200-500	5-200	<5	TOTAL # ITEMS
Premission # Items	2	1	0	1	4	6	16	30
Postmission # Items	2	0	1	1	1	3	3	11

suggested for frozen pre-cooked foods, shellfish and some milk products. (Ref. 22, 23, 24). Corresponding coliform limits are 10 or less per gram. Using these levels as guidelines, the foods tested appear to be of satisfactory quality. A summary of the food sampling is presented in Figure 3-33.

3.5.1 Conclusion

Freeze dried food may be considered microbiologically safe for 90 days.

3.5.2 Recommendations

- Greater quality control should be exercised at the processing plant to ensure uniform bacteria levels.
- Food packages should be dated
- Long-term storage tests should be made at varying temperatures to determine the optimal storage temperature and maximum allowable storage time

3.6 GARMENTS AND LINEN

From the inception of the GSDM, it was recognized that the storage of soiled garments and linen could cause potential problems of odor and bacterial proliferation. It was also apparent that there would not be sufficient storage space onboard to permit a daily change of garments. Therefore, provision was made for a change of underwear every third day and outer garments and linen every seventh day. Antimicrobial treatment of the garments to prevent bacterial proliferation and odor production in the stored soiled garments was suggested.

Initially, laboratory tests were made on swatches of material impregnated with the antimicrobial agent and results indicated a bacteriostatic effect (Figure 3-34). After this, treated and untreated garments were used in a 3-day test dive. Tests of the garments immediately after wearing (i.e., with no storage time) indicated a lower contamination level on the treated items. (Figure 3-36)

Based on this evidence, all linens and garments for the GSDM (with the exception of underwear for Man 6) were treated with the antimicrobial agent. They were packaged

FOOD SAMPLES

	Pre-Mission	Mission	Post-Mission	Total
Total Samples Taken For:	30	N/A	11	41
Microbiology	30	N/A	11	41
Chemical Analysis	0	N/A	0	0
Total Number of Isolates	14	N/A	10	24
Total Number of Sterile Samples	16	N/A	3	19
On Board Readings	N/A	N/A	N/A	N/A
Laboratory Readings	16	N/A	3	19
Total Samples Lost	N/A	N/A	N/A	N/A
in Shipment	-	N/A	-	
in Storage (too long)	-	N/A	-	
Total Number of Isolates Identified to Genus	12(1)	N/A	8(2)	20
Total Number of Genera Found	2(1)	N/A	2(2)	3

(1) plus 1 unidentified gram neg rod

(2) plus 2 unidentified molds

Figure 3-33. Summary: Food Samples

TEST OF ANTIMICROBIAL TREATMENT OF FABRIC

Fabric Type and Treatment	Gram Positive Bacteria			Gram Negative Bacteria			Fungi	
	S. aureus ATCC 6538	B. subtilis ATCC 9466	B. ammoniagenes ATCC 6871	E. coli ATCC 11775	Sal. choleraesuis ATCC 10708	Pseudomonas aeruginosa ATCC 10145	A. niger ATCC 6275	C. globosum ATCC 6205
Cotton Treated With 3% Micro Gard	5 -	7 -	9 -	2 -	3 -	1 -	4 -	3 -
Nylon Treated With 5% Micro Gard	2-3 -	4 -	6 -	1 -	2 -	s -	1 -	1 -
Dacron Treated With 5% Micro Gard	3 -	5 -	7 -	1 -	2 -	1 -	1 -	1 -
Cotton Untreated	O +	O +	O +	O +	O +	O +	OG +	OG +
Nylon Untreated	O +	O +	O +	O +	O +	O +	OG +	OG +
Dacron Untreated	O +	O +	O +	O +	O +	O +	OG +	OG +

NOTE: All Readings Taken After 72 Hrs. for Bacteria, 7 Days for Fungi

5 Zone of inhibition in millimeters
-. No growth under sample on subculture
+ Growth under sample on subculture
OG Over Grown

Test Media: Letheen Agar - Bacteria
Mycophile Agar
With Letheen - Fungi
and Tween

Figure 3-34. Test of Antimicrobial Treatment of Fabric

PRE TEST OF GARMENT TREATMENT EFFECTIVENESS

Garment	Treated With Microgard	Untreated
Undershirt Chest	2	150
Undershirt Right Axilla	15	TNTC
Cotton Knit Shirt	5	TNTC Confluent fungal growth
Under Shorts Groin	27	TNTC
Under Shorts Seat	115	TNTC
Trousers Knee	2	TNTC
Trousers Seat	5	TNTC
Socks Toe	250	TNTC
Socks Sole	52	TNTC

TNTC = Too Numerous To Count

Figure 3-35. Pre-Test of Garment Treatment Effectiveness

and stored in plastic bags until use and then were to be stored in the same bags when soiled. In actuality, not all garments were returned to their individual bags, so post-mission sampling could be done on only those garments which had been properly stored.

Figure 3-36 indicates generally low counts with the exception of the socks and undershorts. It appears, as might be expected, that the items closer to the skin had higher contamination levels. The washcloth had a particularly high count which, in view of its potential for spreading bacteria, must be considered a hazard.

The types of organisms varied, reflecting the men and their environment. Of particular interest is the presence of Pseudomonas and Aerobacter; although it is not surprising in view of their ubiquitous nature on the boat.

It would appear that the treatment of the garments was an effective method for suppressing bacterial growth. The initial 3-day test dive results cannot be considered truly parallel to the GSDM since no storage time was involved. The somewhat higher levels of growth on the stored sample could indicate slow proliferation and an overcoming of the bacteriostatic effects.

A frequency distribution of the bacteria/sq. in. of the 54 samples tested (Figure 3-37) indicates that 40% of the fabrics had approximately 2 organisms per sq. in. Of course this distribution depends on the sample selection. If many more socks and undershorts were included, the curve would be skewed to the right.

The original and main purpose of the linen and garment treatment was the prevention of odor production and bacterial proliferation during storage. However, the overall results of the GSDM indicate that some influence on the resident microbial population of the skin of the crew members may be a secondary effect, and may have contributed to the shift in flora. Establishing a definite cause and effect relationship is complicated by the fact the antimicrobial soaps were used for washing.

The numerous complaints of itching and rashes which were recorded by the crew members in their logs may or may not be related to the antimicrobial agents in the garments, but these should be further investigated, especially as to any adverse effects on the normal protective properties of the skin.

TREATED GARMENTS & LINEN

POST-MISSION ANALYSIS OF STORED ITEMS

GARMENT (NO. OF ITEMS)	LOCATION	CREWMAN	ORGANISMS RECOVERED	NO. ORGANISMS/4 SQ. IN.
Undershirt	Back of Neck	3	Aerobacter	1
(17)	Back of Neck	3	Bacillus, Micrococcus	2
	Rt Axilla	3	Microc., Aero.	5
	Rt Axilla	3	Microc.	2
	Chest		Bac.	1
1st Wk	Back of Neck	4	Microc.	2
2nd Wk	Back of Neck	4	Bac., Microc.	18
7-24	Back of Neck	4	Bac., Microc.	19
7-29	Back of Neck	4	Bac., Microc.	9
1st Wk	Rt Axilla	4	Microc.	4
2nd Wk	Rt Axilla	4	Bac., Aerob.	28
7-24	Rt Axilla	4	Bac.	1
7-29	Rt Axilla	4	Bac.	2
1st Wk	Chest	4	Bac., Microc.	7
2nd Wk	Chest	4	Bac., Microc.	9
7-24	Chest	4	Bac., Microc.	18
7-29	Chest	4	Microc., Pseudo	21
Undershorts	Rt Groin	3	Microc.	3
(12)	Rt Groin	3	Bac., Microc	52
	Seat	3	Microc.	17
	Seat	3	Bac.	1
1st Wk	Rt Groin	4	Bac., Microc.	37
2nd Wk	Rt Groin	4	Bac.	6
7-26	Rt Groin	4	Microc.	TNTC
	Rt Groin	4	Microc.	97
1st Wk	Seat	4	Microc.	84
2nd Wk	Seat	4	Microc.	TNTC
7-26	Seat	4	Microc.	28
	Seat	4		101
Socks	Toe-Bottom	3	Microc., Coryne., Pseudo	TNTC
(5)				
1st Wk	Toe-Bottom	4	Microc.	TNTC
2nd Wk	Toe-Bottom	4	Microc.	10
	Toe-Bottom	4	Microc.	14
	Toe-Bottom	4	Microc.	28

TNTC = Too Numerous to Count

Figure 3-36. Treated Garments and Linen (Sheet 1 of 2)

TREATED GARMENTS & LINEN

POST-MISSION ANALYSIS OF STORED ITEMS (Cont.)

GARMENT (NO. OF ITEMS)	LOCATION	CREWMAN	ORGANISMS RECOVERED	NO. ORGANISMS/4 SQ. IN.
Jump Suit	Rt Axilla	3	Bac., Microc.	4
(7)	Rt Groin	3	Aero.	2
	Seat	3	Aero.	3
1st Wk	Bottom of Neck	4	No Growth	-
	Rt Axilla	4	No Growth	-
	Rt Groin	4	Bac.	1
	Seat	4	Microc.	3
Sheet	Top	3	Microc.	3
(9)	Top		Microc.	1
	Middle		Aero.	1
	Bottom		Microc.	6
	Top		Pseudo, Bac.	9
	Middle		Alternaria	1
	Top, Mid, Bottom	4	No Growth	-
Blanket	Top	5	Aero.	20
(2)	Top	6	Banitratum	24
Washcloth		5	Aero. Pseudo	>1000
Sponge			Aero.	100

Figure 3-36. Treated Garments and Linen (Sheet 2 of 2)

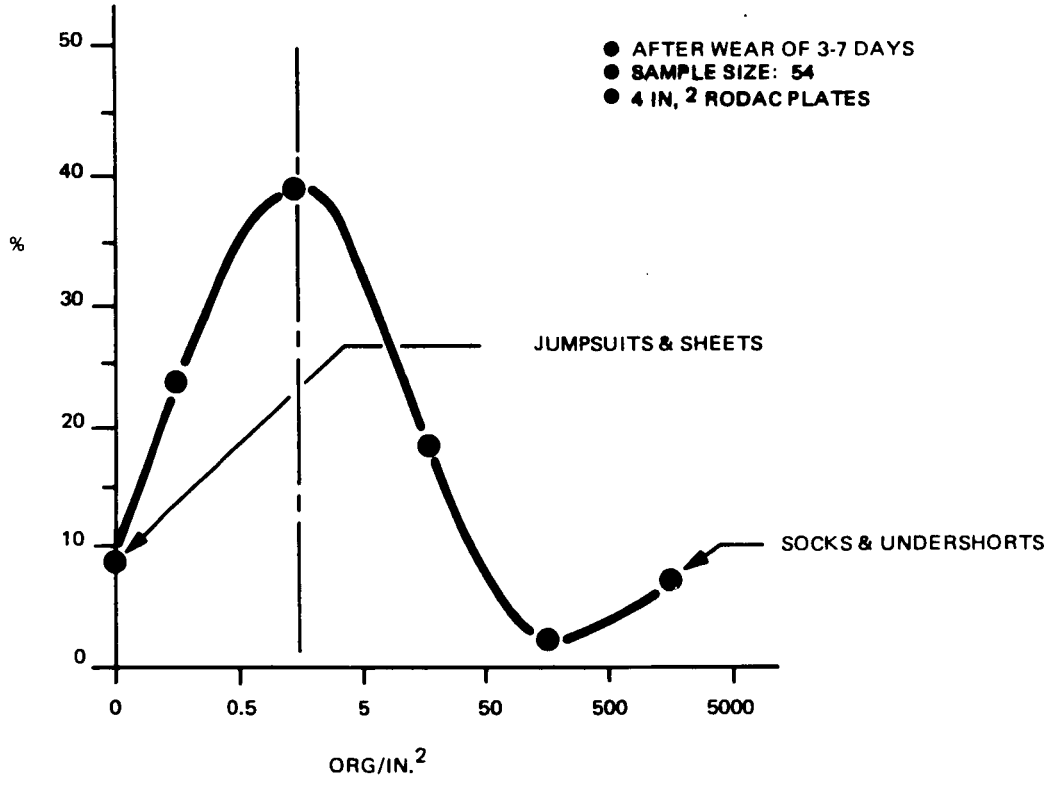

Figure 3-37. Microbial Contamination of Pre-Treated Fabrics

Alternative approaches might be arrived at since the basic idea of garment treatment appears to have merit. Russian experimenters (Ref. 25) have suggested the addition of antibacterial properties to materials used in the manufacture of equipment, clothing, and personal hygiene items for astronauts. However, since antiseptics do not become lastingly attached to materials, they have suggested attaching the antimicrobial agents to the micromolecules of fibrous polymers with a chemical bond.

A summary of the sampling is presented in Figure 3-38.

3.6.1 Conclusions

- Garment treatment is effective in controlling microbial contamination levels and probably odor production of soiled stored garments/linens with the exception of socks and undershorts
- Garments may be subject to the influence of different levels of bacterial loading and dosage of chemical treatments, the time between treatment and use and the length of wear

3.6.2 Recommendations

- Investigate feasibility of post rather than pre-treatment of garments
- Socks and undershorts should have increased chemical treatment, and/or be changed daily
- Conduct controlled experiments with different dosages of treatment and compositions of antimicrobial agents

BEN FRANKLIN GARMENT/LINEN SAMPLES

	Pre-Mission	Mission	Post-Mission	Total
Total Samples Taken For:	20	N/A	54	74
Microbiology	20		54	74
Chemical Analysis	0		0	0
Total Number of Isolates	0		66	66
Total Number of Sterile Samples	20		5	25
On Board Readings	N/A		N/A	0
Laboratory Readings	N/A		N/A	25
Total Samples Lost	N/A		0	0
Total Number of Isolates Identified to Genera	0		66	66
Total Number of Genera Found	0		7	7

Figure 3-38. Summary: Ben Franklin Garment/Linen Samples

SECTION 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

- The GSDM demonstrated that man was capable of existing without serious illness in biological isolation for 30 days, using a simple life support system under conditions in the BEN FRANKLIN
- An apparent shift and simplication of microbial flora did occur during biological isolation, in the direction of the gram negative rods. At the end of 30 days equilibrium had not been reached
- As the mission progressed, the flora found on the crew and in their environment became similar, including the shift and simplication
- The personal hygiene area (particularly the shower) was the most contaminated environment location in spite of repeated antimicrobial treatment
- The techniques used to monitor the water contamination were inadequate. Potability in the cold water loop was rapidly lost. It appears that at least one route of water contamination was from the outside (taps) inward.
- As designed and operated, the waste management system was inadequate from a microbiological point of view
- The use of antimicrobials influenced the microbial ecology. While some temporary advantages appeared to accrue, their overall effect seemed to be undesirable

4.2 RECOMMENDATIONS

- Formulate an experiment to further delineate the phenomenon of flora shift and simplification
- Re-evaluate cleansing protocols
- Redesign the water management system with biocompatible materials and with provisions to prevent contamination from external sources
- Re-evaluate and redesign the waste system

- Refine and automate the onboard microbial monitoring system
- Perform a thorough evaluation of antimicrobial agents including:
  - Use versus non-use
  - Comparison of various agents
  - Pre-versus post-wear garment treatment.

4.3 GUIDELINES FOR FUTURE SPACE STATIONS BASED ON GSDM EXPERIENCE

- Attempt to maintain a balanced microbial flora
- Design for effective "house cleaning", with particular attention to personal hygiene area
- Select compatible materials and chemicals
- Design waste management system to permanently deactivate waste materials
- Use other means in addition to filters for microbial control
- Develop automated on-line contaminant monitoring
- Provide means for effective decontamination.

SECTION 5

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APPENDIX A

MANNED CHAMBER TESTS

Program		Year	Crew	Duration Days	Relative Biological Isolation*	Remarks and Results
Sch. Aerospace Med. NASA	I	1962	2	14	2	1/3 ATM 100% O_2 No Bio.
	II					
	III	1967		56	2	1/3 ATM 100% O_2
Republic Aviation	I	1962	6	14	1	1 ATM 100% O_2 Ext. Bio.
NASA	II	1962	6	14	1	1/2 ATM 100% O_2 Ext. Bio.
	III	1962	6	14	1	1/3 ATM 100% O_2 Ext. Bio.
	IV	1962	6	14	1	1/4 ATM 100% O_2 Ext. Bio.
Naval Air Equip. Lab. NASA	Crew/I	1963	6	14	2	1/3 ATM 100% O_2
Sea Lab. Navy	I	1963	4	14	2	6 ATM He/O_2 Little Bio.
Sea Lab Navy	II	1965	3 crews 10 each	Total 45 days 15 days/crew	1	6 ATM He/O_2
Wright/Pat. NASA/AF	I	1965	4	45	2	1 ATM N_2/O_2
	II(1)		4	42	2	
	(2)		4	43	2	
	(3)		4	43	2	
	(4)		4	43	2	
	(5)		4	46	2	
	III(1)	1966	4	42	2	
	(1A)	1966	3	21	2	
	(2)	1966	4	60	2	
Boeing Mesa (NASA)	I	1965			2	
	II	1965			2	
McDonnell		1968			3	1/3 ATM 100% O_2

* 1 = least isolated
6 = most isolated

Program	Year	Crew	Duration Days	Relative Biological Isolation*	Remarks and Results
Douglas NASA NASA Langley	1968	4	3 shifts of 4 men each day 28	1	1/3 ATM 100% O_2 No Sign. Bio.
Tektite Navy/NASA/G.E.	1969	4	60	2	2 ATM N_2/O_2 Ext. Bio.
Ben Franklin Navy/NASA/Grumman	1969	6	30	6	1 ATM N_2/O_2 Ext. Bio.

* 1 = least isolated
  6 = most isolated

APPENDIX B

WATER CHEMISTRY

The research submersible, BEN FRANKLIN had four vacuum-jacketed tanks for hot ($90^{o}C$) water storage with a total capacity of about 180 gallons, and four slightly larger tanks with an overall capacity of 350 gallons for cold water storage. These were the only sources of water during the GSDM; therefore, it was essential that adequate water quality be maintained if the crew's well-being was to be adequately safeguarded. This was done by initially providing high quality water* that was carefully handled and stored in a system that had been very thoroughly pre-cleaned.

The hot water was to be used for the reconstitution of the freeze-dried foods and for the preparation of hot drinks. The cold water was intended for all other purposes, including showers and waste management system operation.

The pre- and post-mission chemical analyses of the water are presented in Figure B-1 and B-2. Water potability standards are presented in Figure B-3. The trends obtained from the pre-mission data can be applied to data pertaining to the water supplied for the mission. Since the general trends were fairly similar, most of the data for the cold water system were obtained at the galley sink. The analytical procedures employed were those specified in "Standard Methods for the Examination of Water and Wastewater," although a few were automated versions adapted for use with an auto analyzer.

In all cases, the pH tended to increase by one to three units (usually one to two) with time. All of the water was mildly to slightly acidic (3.2 pH 6.9), except for a few samples hovering in the 7.1 to 7.3 pH range. The hot water tanks became moderately alkaline (up to 9.6 pH) during the mission. Electrical conductivity usually ran less than 100 micromhos and

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*Sterility is discussed elsewhere.

WATER ON BOARD (Sample #293-303 & 314-322)

Date	Sample Location	Sample #	ppm Chloride	ppm Ammonia	ppm Phosphate	ppm Nitrate	ppm COD	ppm Iodine	JCU Turbidity	Chloropl Color	mho Conductivity	pH	TGE Count
7-14	Galley Sink Tank 1	293	3.2	0.01	0.1	0.45	1212	0.5	5.0	2.9	90	6.60	120/290
8-22	Galley Sink Tank 1	314	1.8	0.01	0.1	0.25	1725	-	5.0	1.4	70	6.22	59,400
7-14	Head Sink Tank 1	294	5.6	0.01	0.1	1.2	2697	0.3	9.5	0.0	90	5.99	
8-22	Head Sink Tank 1	315	1.8	0.01	0.1	0.25	1820	-	0.0	8.3	70	6.38	30,250
7-14	Shower Sink Tank 1	295	30.0	0.08	0.3	0.4	1029	0.75	43.5	4.5	230	6.61	
8-22	Shower Sink Tank 1	316	1.6	0.01	0.1	0.85	1782	-	0.0	4.7	80	6.39	24,200
7-14	Shower Dispenser Tank 1	296	2.0	0.08	0.2	0.5	1626	0.4	2.5	0.7	90	7.30	
8-22	Shower Dispenser Tank 1	217	1.8	0.01	0.1	0.3	1637	-	4.5	0.7	70	6.08	11,000
7-14	Galley Sink Tank 2	297	1.8	0.01	0.1	0.5	1686	0.2	2.9	1.2	80	6.70	7,400
8-22	Galley Sink Tank 2	318	7.0	0.03	0.1	0.25	1784	-	4.8	0.7	90	6.71	11,870
7-14	Galley Sink Tank 3	298	1.0	0.01	0.1	0.3	1649	0.2	12.0	1.4	80	6.68	25
8-22	Galley Sink Tank 3	319	5.0	0.05	0.1	0.35	1778	-	2.0	2.8	90	6.88	25
7-14	Galley Sink Tank 4	299	1.0	0.04	0.15	0.5	1561	0.7	12.0	0.0	80	6.80	3
8-22	Galley Sink Tank 4	-	-	-	-	-	-	-	-	-	-	-	-
7-14	Hot Water Tank 1	300	0.1	0.01	0.15	0.1	6.8	-	44.8	0.1	90	9.20	0
8-22	Hot Water Tank 1	-	-	-	-	-	-	-	-	-	-	-	-
7-14	Hot Water Tank 2	301	0.1	0.01	0.1	0.1	6.8	-	7.0	0.0	100	9.00	0
8-22	Hot Water Tank 2	320	0.3	-	-	0.1	2.0	-	-	-	-	-	14,300
7-14	Hot Water Tank 3	302	0.1	0.01	0.1	0.1	2.0	-	10.0	010	95	9.10	0
8-22	Hot Water Tank 3	321	0.2	0.01	0.15	0.1	9.56	-	49.0	1.0	220	8.22	330
7-14	Hot Water Tank 4	303	0.1	0.01	0.1	0.1	2.0	-	3.0	0.0	100	9.00	0
8-22	Hot Water Tank 4	322	0.1	0.01	0.1	0.1	2.0	-	3.0	0.7	140	8.22	346

Figure B-1. Water On Board

POST-MISSION SAMPLING

Date	Sample Location	Sample #	ppm Chloride	ppm Ammonia	ppm Phosphate	ppm Nitrate	ppm COD	ppm Iodine	JCU Turbidity	Chloropl Color	mho Conductivity	pH	TGE Count
8-22	Galley Sink Line Tank #1	310	0.8	0.05	0.1	0.25	1,725	-	5.0	1.5	90	6.40	16,500
8-22	Head Sink Line Tank #1	311	1.2	0.04	0.15	0.30	1,382	-	11.3	0.7	90	6.42	77,000
8-22	Shower Sink Line Tank #1	312	3.0	0.04	0.1	0.25	1,695	-	21.0	0.7	110	6.42	17,600
8-22	Shower Sink Dispen. Line Tanks #2 & 4	313	1.4	0.01	0.1	0.25	1,790	-	8.5	0.0	95	6.56	6,600
8-22	Galley Sink Tank #1	314	1.8	0.01	0.1	0.25	1,725	-	3.0	1.9	70	6.22	59,400
8-22	Head Sink Tank #1	315	1.8	0.01	0.1	0.25	1,820	-	0.0	2.3	70	6.38	30,250
8-22	Shower Sink Tank #1	316	1.6	0.01	0.1	0.25	1,782	-	0.0	4.7	80	6.39	24,200
8-22	Shower Dispenser Tank #1	317	1.8	0.01	0.1	0.30	1,637	-	4.5	0.7	70	6.08	11,000
8-22	Galley Sink Tank #2	318	7.0	0.03	0.1	0.25	1,784	-	4.8	0.7	90	6.71	11,870
8-22	Galley Sink Tank #3	319	5.0	0.05	0.1	0.35	1,778	-	2.0	2.8	90	6.88	25
8-22	Hot Water Tank #2	320	0.3	Ins.	0.1	0.1	2.0	-	Ins.	Ins.	Ins.	Ins.	14,300
8-22	Hot Water Tank #3	321	0.2	0.01	0.15	0.1	9.56	-	49.0	1.0	220	8.22	330
8-22	Hot Water Tank #4	322	0.1	0.01	0.1	0.1	2.0	-	30.0	0.7	140	8.22	340
8-22	Bilge Water	323	1640	1180	475	3.9	14,531	-	7000	700	7,000	7.22	TNTC 10^6
8-22	Mini-Waste Tank (Bottom of Tank)	324	1260	1040	740	10.0	20,693	-	7000	790	9,250	7.06	1.1×10^6
8-22	Mini-Waste Tank (Middle of Tank)	325	1260	1120	500	5.1	19,307	-	7000	500	9,500	7.05	9.6×10^6
8-22	Mini-Waste Tank (Top of Tank)	326	1260	1160	500	5.1	19,230	-	7000	600	9,250	7.09	2.0×10^7
8-22	**Main Waste Tank #1 (Bottom)**	327	1080	1320	560	5.1	19,597	-	900	800	10,300	7.03	9.0×10^6
8-22	**Main Waste Tank #1 (Middle)**	328	1080	1280	560	5.0	19,481	-	800	1000	10,400	7.08	1.3×10^7
8-22	Main Waste Tank #2 (Bottom)	329	860	1296	720	5.0	19,307	-	7300	800	10,000	7.02	6.5×10^6
8-22	Main Waste Tank #3 (Bottom)	330	1140	1280	550	3.8	19,497	-	8200	800	10,400	7.01	1.2×10^7
8-22	Main Waste Tank #4 (Bottom)	331	1020	1408	550	5.0	25,590	-	6400	640	9,900	7.02	1.1×10^7

Figure B-2. Post-Mission Sampling (Sheet 1 of 2)

POST-MISSION SAMPLING (Continued)

Date	Sample Location	Sample #	ppm Chloride	ppm Ammonia	ppm Phosphate	ppm Nitrate	ppm COD	ppm Iodine	JCU Turbidity	Chloropl Color	mho Conductivity	pH	TGE Count
8-22	100 ppm Sterilizing I_2	332	-	-	-	-	-	-	770	770	-	2.62	-
8-22	1% Iodine	333	-	-	-	-	-	-	770	770	-	2.31	-
8-22	Galley Sink Trap	334	7600	124	75	Ins.	4,731	-	Ins.	Ins.	Ins.	Ins.	4.3×10^8
8-22	Head Sink Trap	335	35	56	64	0.25	2,951	-	770	Ins.	590	6.95	6.0×10^8
8-22	Shower Sink Trap	336	Ins.	Ins.	Ins.	Ins.	Ins.	-	Ins.	Ins.	Irs.	Ins.	10^6
8-22	Toilet Water	337	1300	760	550	0.3	11,805	-	770	Ins.	8,750	7.95	4×10^4
8-26	Galley Filter Bowl	338	Ins.	Ins.	Ins.	Ins.	1,417	-	Ins.	Ins.	Ins.	Ins.	4.1×10^3
8-26	Head Filter Bowl	339	Ins.	Ins.	Ins.	Ins.	1,249	-	Ins.	Ins.	Ins.	Ins.	10^6
8-26	Shower Filter Bowl	340	3.75	0.08	0.2	0.17	1,647	-	420	1.8	110	6.65	10^6
7-30	Galley Sink	341	1.8	0.02	0.15	0.15	806	-	Ins.	Ins.	90	6.95	4.7×10^6
7-30	Head Sink	342	1.65	0.06	Ins.	0.13	825	-	Ins.	Ins.	Ins.	6.08	3.8×10^7
7-30	Shower Sink	343	1.15	0.01	0.05	0.23	1,109	-	3.0	Ins.	50	6.15	1.4×10^5
7-30	Shower Sink	344	1.5	0.01	0.05	0.25	1,113	-	Ins.	Ins.	Ins.	Ins.	1.1×10^5

Figure B-2. Post-Mission Sampling (Sheet 2 of 2)

WATER POTABILITY STANDARDS

	Cl^-	NH_4	PO_4	NO3	COD ppm	TDS ppm	TURB	COLOR	CONDUCT	pH	TOTAL COUNT	COLIF/ 100 ml
USPHS	250			45		500	5	15				1
WHO	350	0.5		50		1500						0.5
NAS-NRC	450			10	100	1000	10	15			0	0
GRUMMAN	250	0.5		45	50	800	15	15	1700	5-8	0	0
NASA C-35						500	5	15		6-8	0	0
NASA PF-1B						14	5	CM LM 15 100		6-8	0	0

Figure B-3. Water Potability Standards

fell off slightly with time to less than 50 micromhos (our limit of detection), except for an apparent elevation in cold water tanks 2 and 3 during the mission. Heavy metals (Ni, Cu, Cr, Ag, Hg, Fe, Pb, and As) analyses are being performed by atomic absorption spectroscopy; these results will be reported at a later date.

Both color and turbidity decreased with time during the third fill cycle (23 to O JCU, and 18 to O CPU)* and the GSDM (12 to 2 JCU, and 3 to 0 CPU). They remained essentially constant (1 to 3 JCU and CPU) or rose slightly (to 5 JCU) with time during the fourth fill cycle. Post-mission samples from the bilge and the waste tanks ran as high as 1000 CPU (by serial dilution of samples). As expected, the iodine concentration fell off rather rapidly with time by as much as three orders of magnitude (10 to 0.01 ppm) within 12 days.

There was no appreciable change in the concentrations of ammonia-nitrogen (typically, 0.1 to 0.3 ppm), or phosphate (typically, 0.1 ppm) other than an occasional random perturbation. However, they were significantly elevated in the post-mission bilge water (1180 ppm to NH_4 and 475 ppm for PO_4) and in the waste tanks (1200 and 600 ppm average for NH_4 and PO_4, respectively).

For the most part, the chloride ion content held fairly steady (concentrations ranged from 0.7 to 9 ppm) or dropped slowly in an irregular fashion. In no case, did the overall excursion of chloride concentrations in the water supply exceed much more than one order of magnitude; however, the bilge and waste tanks were another matter. Chloride ion concentration in the waste tanks typically ran higher than 1000 ppm. It was more than 7000 ppm in the galley sink trap which was, of course, to be expected. But the bilge contained more than 7600 ppm chloride, which was significantly greater than anticipated and much above any other source. There is no ready explanation for this phenomenon, since the vessel was sealed and did not have any apparent, or readily detectable, leaks.

Chemical oxygen demand (COD) remained fairly constant and was usually in the region of 1000 ±300 ppm, except for the hot water tanks (which were all less than 7 ppm for the water). It was interesting to note that the post-mission COD of the bilge water was more than 14,000 ppm, although the post-mission water tanks did not exceed 1800 ppm. The

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* JCU - Jackson Candle Units; CPU - Chloroplatinate Units

COD of the waste tanks ranged from 19,200 to 25,600 ppm; the toilet water was a mere 11,800 ppm. The COD in the galley sink trap was much lower (only 4700 ppm) than might be expected. Eight days post-mission (38 days after loading), the COD content of hot water tanks 2 and 4 were each still less than 2.0 ppm; hot water tank 3 was only 9.6 ppm (Tank 1 was dry).

Particulate analyses were run with the Particle Data Celloscope and a pulse height analyzer. These data showed occasional loadings of particles in the 0.6 to 1.5 micron size range; however, initial attempts to correlate them with microbial plate counts on TGE agar were unsuccessful. Instrumentation problems precluded obtaining much data on the larger particulates (to 100 microns).

APPENDIX C

ON-BOARD SAMPLING INSTRUCTION MANUAL

C-1 PROCEDURE FOR TAKING WATER SAMPLES FOR MICROBIAL CONTAMINATION DURING THE GULFSTREAM DRIFT MISSION

I Scope:

This test procedure will be used for the verification of Water Potability.

II. Purpose:

Water Potability is a medical safety requirement. This procedure provides a field test, to monitor the water for Microbial Contamination, and Iodine Concentration.

III Facilities and Test Equipment:

- 1 Millipore all metal syringe with two way valve.
- 1 Stainless steel beaker, graduated.
- 200 Bacteriological field monitors.
- 1 Box of Endo Media (in ampules).
- 1 Box of Total Media (in ampules).
- 1 Box of Yeast-Mold Media (in ampules).
- 1 Box of sterile sample tubes.
- 2 Bottles of 70% Ethyl Alcohol
- 1 Iodine Test Kit consisting of:
  - 1 Colorimeter (color compairator) with sample tubes.
  - 1 Bottle of Iodine Reagent (0.2% 0-Tolidine).
  - 1 Bottle of Complexer (set. sol. Hg_2 Cl_2).
  - 1 Graduated cylinder, 25 ml.

IV. Methods:

At the time of biological sampling the cold water should be checked for Iodine Concentration.

V Procedures:

Microbial Test

$HgCl_2$ AND ORTH. ARE TOXIC AND SHOULD NOT BE TAKEN INTERNALLY. WASH HANDS AFTER USE.

The stainless steel beaker is rinsed with 70% Ethyl Alcohol. Just enough alcohol is used to cover the inner surface while rinsing (approx. 5-10 mls.) with a rotary motion. The alcohol is discarded and the beaker is rinsed 3 times with the water to be tested to remove any residual alcohol.

The beaker is aseptically filled with the water to be tested.

A Millipore Bacteriological field monitor is placed on the inlet side of the syringe with the filter side out (filter is ruled into squares), as per instructions printed on the syringe. They read:

"The Luen Taper connection of the valve shall be attached to the outlet hole of a field monitor."

The end of the sampling tube is inserted into the test water and the plunger of the syringe is slowly pulled back as far as it will go. The plunger is then pushed forward discharging the filtered water to waste. This pumping is repeated until 100 mls. of water is removed from the beaker.

This procedure is repeated with two other monitors, making a total of 3 monitors for each water sample.

After the 3 monitors have been used they are placed filter side down.

An ampule of media is taken from the media carton and the tip is broken off (the bare end of the ampule) the end (broken) is gently placed into the hole of the monitor (where the syringe had been attached) and the top of the ampule (with the plastic tubing) is snapped with the fingers, thus allowing the liquid media to saturate the backing pad in the monitor.

One monitor is treated with Endo Media, one with Total Media and one with Yeast-Mold Media.

The end plugs are replaced into the monitors.

Each monitor is labeled as to source of water (i.e., galley sink cold water tank #1, 1400 hrs. 4/23/69, Endo) time, date and media.

They are then stored filter down.

The monitors are observed at 24 hr., 48 hr. and 72 hrs. for signs of growth. If growth occurs it will appear as small raised, circular colonies on the filter surface. The number of colonies are recorded on the data sheet. In the event of gross contamination the number of colonies may be too numerous to count (TNTC), or the colonies may merge into each other giving a smeared appearance or may appear as confluent growth.

Growth on Endo Media is indicative of coliform bacteria. If this occurs the results should be reported to the doctor or medical staff member immediately.

All monitors and records should be stored unopened and sent to the Biotechnology Laboratory, Plant 31, Bethpage, New York.

NOTE: The filter faces the sample!

Not applicable

Not applicable

IODINE TEST

Take 25 mls. of the water to be tested

Add 1 drop of Complexing Solution (sat. Hg_2 Cl_2)

Add 0.5 mls. of Iodine Reagent (0.2% 0- Tolidine)

Shake to mix and fill Colorimeter tube

Compare color of test solution to standard

Record PPM (Parts per million) Iodine on data sheet

C-2 PROCEDURE FOR THE TAKING OF ENVIRONMENTAL SAMPLES FOR MICROBIAL CONTAMINATION DURING THE GULFSTREAM DRIFT MISSION

I Scope:

This procedure will be used for the determination of Microbial Contaminants in the biological isolated environment of the BEN FRANKLIN.

II. Purpose:

Microbial contamination in the ecologically closed environment can have direct bearing on the health of the health of the crew. It is essential to monitor the rise and/or fall of such contaminants in order to determine their sources and to develop adequate controls.

III Facilities and Test Equipment:

1 Andersen Microbial Air Sampler

60 Nutrient agar plate for Andersen Sampler

250 Rodac plates with Letheen agar

2 Rolls of plastic tape

IV Procedure:

Air Sample

Not Applicable

The Andersen Sampler is disassembled and wiped down with 70% Ethyl Alcohol.

Not Applicable

Not Applicable

The sampler is re-assembled asceptically placing the lower half of a Petri dish containing sterile nutrient agar between each stage of the sampler. The tops of the Petri dishes are set aside (so as not to become contaminated) until after the sample is taken.

The spring clips are fastened.
(the sampler is now ready for use)

The sampler is placed on location and power switch is turned on. Sampling time is 5 min.

The sampler is disassembled starting with the top stage (1) as the Petri dish is exposed the Petri dish cover is replaced on it. The same process is used for all 6 stages.

The Petri dishes are sealed with tape.

All Petri dishes are observed at 24, 48 and 72 hrs.

The number of colonies is recorded for each plate.

Fomites:

Sixteen locations are sampled every third day, using Rodac plates containing Letheen agar.

The cover of the Rodac plate is removed and held in the left hand, the bottom is held by the finger tips of the right hand.

The agar surface of the Rodac plate is placed on contact with the surface to be sampled with a slight rolling motion (like used in finger printing).

The cover is replaced and labeled and taped.

The surface sampled is cleaned (with 70% alcohol) to remove any traces of media residue.

The plates are examined at 24, 48 and 72 hrs.

The number of colonies are counted and recorded at that time.

C-3 PROCEDURE FOR TAKING HUMAN FLORA SAMPLES FOR MICROBIAL FLORA, DURING THE GULFSTREAM DRIFT MISSION

I Scope:

This sampling procedure will be used for the determination of the micro flora of the BEN FRANKLIN crew during the drift mission.

II. Purpose:

The microbial flora of the crew and its changes will be monitored by the sampling procedure. Samples will be taken every 3rd day.

III. Facilities and Test Materials:

500 Sterile swabs

500 Carry and Blair transport medium

200 Rodac plates with Blood AGAR

30 Swubes sample tubes

30 Sterile 30 ml bottles

IV. Procedures:

Sampling will be performed every 3rd. day prior to showering, bathing or otherwise cleansing of the body, and before changing of undergarments.

Test Procedure for Part I

SWAB SAMPLES

The sterile swab is aseptically removed from the protective package

The area to be sampled is exposed and the sample taken with a gentle rolling motion of the swab.

The cap is removed from the carry and Blair transport media asceptically

The end of the swab (cotton end) is inserted into the tube and broken off, aseptically (remaining swabstick is discarded)

The cap is replaced and sealed with tape

The sample tube is labeled and placed into storage

RODAC PLATE SAMPLES (SURFACES)

The cover of the Rodac Plate is removed with the finger tips of the left hand.

The base of the Plate is held in the finger tips of the right hand aseptically

The AGAR surface of the Plate is placed in contact with the surface to be sampled with a slight rolling motion.

The cover is replaced

The Rodac Plate is labeled and stored

NOTE: The stored samples will be sent to the Biotechnology Lab., Plant 31, Bethpage, New York for analysis at the earliest convenience at the end of the drift.

Urine Samples (to be taken by individual crew members)

Samples shall be collected from the first voiding after sleep.

Urine samples will be taken once a week before the operation of the SAS unit.

These samples will be sent to the surface via the SAS.

Remove cap aseptically from the sample tube

Take aim and fill sample tube with urine

Replace cap, label, and seal with tape

Place in SAS ball.

Fecal Sample (to be taken by individual crew members)

At the time of defecation, during the day prior to the operation of the SAS unit, Fecal samples shall be taken by each of the crew members.

Defecate

Remove paddle from tube aseptically

Take sample and replace paddle with sample in tube

Label and seal with tape

Place in SAS ball.